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ENDOSCOPIC TREATMENT OF MINOR BILIARY INJURY, PREVENTION AND EASY DIAGNOSIS OF POST-ERCP PANCREATITIS, AND PREDICTION OF SEVERE ACUTE PANCREATITIS

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ABSTRACT

The primary therapeutic options in minor biliary injury (BDI) after cholecystectomy are endoscopic sphincterotomy (ES) only or with stenting (EST) depending on the type and severity of injury.

Non-steroidal anti-inflammatory drugs (NSAIDs) based mostly on studies in high risk patients are recommended agents in prevention post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis (PEP). The diagnosis is traditionally based on plasma or serum amylase analyses. The feasibility of an easier and quicker urine trypsinogen-2 (T-2) dipstick method in ERCP unit has not been properly explored, hampering its utilisation.

Acute pancreatitis (AP) initiating of different aetiologies varies in severity, from mild AP resolving within days to a severe form (SAP) with organ dysfunction (OD) and high morbidity. Most ODs develop after hospital admission. Early recognition of these patients would allow the initiation of maximal intensive care.

The aims of this study were (I) to explore whether ES or ES with stenting is superior in minor (Amsterdam type A) bile duct leaks, (II) to evaluate if rectal diclofenac has a prophylactic effect in PEP in an ERCP unit with low PEP rate, (III) to explore the urine T-2 dipstick test in detecting PEP, and (IV) to explore whether serum SPINK1, trypsinogens 1 to 3 (T-1, T-3) and a complex of trypsin-2 and α_1 -antitrypsin (trypsin-2-AAT), can predict the development of SAP in patients without OD at hospital admission.

In these four clinical studies, all the patients were referred to Helsinki University Hospital (HUH) Abdominal Centre between 2004 and 2018. In retrospective study I, 71 ERCP-patients with minor bile leak (Amsterdam type A) and native papillae were grouped into ES group (ES group, n=50) and ES with stenting group (EST group, n=21). In retrospective study II, 1,000 ERCP patients with 100 mg rectal diclofenac formed the diclofenac group (DG), and 1,000 patients without rectal diclofenac served as a control group (CG). In prospective study III, 400 ERCP patients with native papilla and without AP were tested with a dipstick test before, and 4 and/or 24 h after ERCP. In prospective study IV, 239 patients admitted to the HUH emergency room due to AP, SPINK1, T-1, T-2 and T-3, and a trypsin-2-AAT, plasma pancreas specific amylase or amylase, creatinine, and C-reactive protein (CRP) were measured 0-12 h after admission to hospital.

Study I revealed no difference in outcomes in the ES and EST groups in the closure time of the leak, discharge time from hospital, and in the primary healing rate in a high- or low-grade leak. In study II, the incidence of PEP was 2.8% in both DG and CG groups, and there was no difference between the groups in the severity of PEP or in the effect of diclofenac in higher-risk subgroups. In study III, a urine T-2 dipstick test in the diagnosis of PEP was highly accurate when the test was evaluated with abdominal pain symptoms;

the sensitivity, specificity, positive and negative predictive values 4 and 24 h after ERCP were 60%, 99%, 71%, 98% and 100%, 98%, 71%, 100%. In study IV, serum levels of SPINK1, T-1, T-2, trypsin-2-AAT, and creatinine correlated on admission with the severity of AP. SPINK1 was the most accurate predictor for development of SAP, followed by T-2.

In conclusion, ES seems to be equal to ES and stenting in the treatment of Amsterdam type A bile duct leaks. In a centre with a low risk of PEP, rectal diclofenac showed no preventive effect. In diagnostics of pancreatitis, a negative urine T-2 dipstick test rules out PEP 4h after ERCP, while a positive test with abdominal pain symptoms accurately reveals PEP. In assessing AP patients without OD at the time of hospital admission, SPINK1 appears to be a useful predictor of SAP.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which in the text are referred to by their Roman numerals:

I Rainio M, Lindström O, Udd M, Haapamäki C, Nordin A, Louhimo J, Kylänpää L. Endoscopic therapy of Biliary injury. Digestive Diseases and Sciences. 63:474-480, 2018

II Rainio M, Lindström O, Udd M, Louhimo J, Kylänpää L. Diclofenac Does Not Reduce the Risk of Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis in Low Risk Units. Journal of Gastrointestinal Surgery. 21:1270-1277, 2017

III Rainio M, Lindström O, Udd M, Tenca A, Puolakkainen P, Stenman U-H, Kylänpää L. Urine trypsinogen-2 dipstick test in diagnosis of post-ERCP pancreatitis (submitted)

IV Rainio M, Lindström O, Penttilä A, Itkonen O, Kemppainen E, Stenman U-H, Kylänpää L. Serum SPINK, trypsinogens 1-3, and complex of trypsin-2 and α_1-antitrypsin in the diagnosis of severe acute pancreatitis. Pancreas. 48(3):374-380, 2019

ABBREVIATIONS

AP	acute pancreatitis
APACHE	acute physiology and chronic health evaluation
AUC	area under the ROC curve
BDI	bile duct injury
CARS	compensatory anti-inflammatory response syndrome
CECT	contrast-enhanced computed tomography
CRP	C-reactive protein
CG	control group
DAMP	damage-associated molecular pattern
DOR	diagnostic odds ratios
DG	diclofenac group
ERCP	endoscopic retrograde cholangiopancreatography
ERC	endoscopic retrograde cholangiography
ES	endoscopic sphincterotomy
EST	endoscopic sphincterotomy and stenting
ESGE	European Society of Gastrointestinal Endoscopy
FC-SEMS	fully covered self-expandable metal stent
HUH	Helsinki University Hospital
IL	interleukin
MRI	magnetic resonance imaging
MMS	modified Marshall score
NF-κB	nuclear factor κB
-LR	negative likelihood ratio
NPV	negative predictive value
NSAIDS	non-steroidal anti-inflammatory drugs
OR	odds ratio
OD	organ dysfunction
PCT	procalcitonin
PEP	post-ERCP pancreatitis
PLA2	phospholipase A_2
PPV	positive predictive value
PSTI	pancreatic secretory trypsin inhibitor
PTC	percutaneous transhepatic cholangiography
+LR	positive likelihood ratio
ROC	receiver-operating characteristic
RCT	randomised clinical trial
SAP	severe acute pancreatitis
SPINK1	serine peptidase inhibitor Kazal-type 1
SIRS	systemic inflammatory response syndrome

Su-PAR	soluble urokinase-type plasminogen activator receptor
trypsin-2-AAT	trypsin-2 complexed with α_1-antitrypsin
T-1	trypsinogen-1
T-2	trypsinogen-2
T-3	trypsinogen-3
TATI	tumour-associated trypsinogen inhibitor
US	ultra sound
WON	walled-off necrosis

1 INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is a diagnostic and therapeutic endoscopic procedure first described in 1968, and over the decades it has been developed as a specific tool for pancreatic and biliary system disorders. It is efficient in the therapeutics of post cholecystectomy biliary duct injury (BDI), which appears as leaks and strictures in the biliary tree (Bergman et al., 1996). Treatment options for BDI, depending on type and severity of trauma, are endoscopic sphincterotomy (ES) alone in some cases of bile leaks, or stenting with or without ES in major leaks and all strictures. The most common type of BDI is a leak, and most frequently, in 60-78% of cases, the site of the leak is found in the cystic duct remnant (Kaffes et al., 2005, Sandha et al., 2004). The European Society of Gastrointestinal Endoscopy (ESGE) guideline recommends endoscopic placement of plastic stents in the management of minor bile duct leaks (J. M. Dumonceau et al., 2018).

Post-ERCP pancreatitis (PEP) is the most common and feared complication of ERCP, resulting in complex and multifactorial reactions in the pancreatic gland due to pancreatic duct opacification, guide wire passages, and other instrumentation (Messmann et al., 1997). Despite improvements in ERCP techniques and equipment, the incidence of PEP has not significantly improved. Studies report incidences of PEP between 2-9% and severe PEP between 0.3- 0.6% (Freeman et al., 2004, Andriulli et al., 2007, Ding et al., 2015). Many pharmacological agents have been investigated in PEP prevention, with poor results. Currently, non-steroidal anti-inflammatory drugs (NSAIDs) seem the most efficient and studied agent in PEP prevention (Yu et al., 2018). ESGE guidelines suggest routine use of rectal NSAIDs for all ERCP patients without contraindications (J. Dumonceau et al., 2014).

Traditionally, PEP diagnosis has been based on measurements of serum or plasma amylase or lipase. However, after insult of PEP, trypsinogen-2 (T-2) concentrations in urine rapidly increases. A fast dipstick test for urine T-2 has been developed and it has been shown to be equal to amylase and lipase measurements in diagnosis of acute pancreatitis (AP) and PEP. (Hedstrom et al., 1996, M. Kylanpaa-Back et al., 2000, Rompianesi et al., 2017) Only three studies have evaluated the use of trypsinogen-2 dipstick test in the diagnosis of PEP. The lack of studies may have inhibited the utilisation of the dipstick test in ERCP units.

The course of AP varies, and in the severe form of AP, the development of organ dysfunction (OD) can be rapid, leading to multiple organ failure and even death. It is crucial to identify those patients prone to developing OD to provide optimal treatment in the intensive care unit. Several biomarkers and scoring systems have been developed to measure the severity of AP and predict the development of OD. None has been sufficiently reliable, and the estimation of which of the AP patients need intensive care is based on the clinician's

evaluation. A trustworthy laboratory test that would help in the assessment of the development of OD is still lacking.

In this thesis, we investigated ERCP in the treatment of BDI after cholecystectomy and in especially whether ES or ES and stenting is superior in minor, Amsterdam type A BDI leaks. Secondly, we focused on PEP, which is a rather frequent complication of ERCP. We evaluated the rectal diclofenac preventive effects on PEP in a retrospective cohort of 2,000 patients in our low PEP rate ERCP unit. We also explored the diagnostics of PEP by examining the urine T-2 dipstick test in 400 patients with native papillae. To investigate further the diagnostics of AP and especially severe AP, we examined in 238 patients referred to the emergency room whether serum SPINK1, trypsinogens 1 to 3, and complex of trypsin-2 and α_1 -antitrypsin could predict the development of OD in patients who presented no signs of OD on arrival at the hospital.

2 REVIEW OF THE LITERATURE

2.1 ERCP

Endoscopic retrograde cholangiopancreatography (ERCP) is a diagnostic and therapeutic procedure for pancreatic and biliary system disorders. The term cholangiopancreatography describes a radiological procedure in which biliary and pancreatic ductal systems are cannulated and injected with contrast material to gain diagnostic information. Currently, ERCP is mainly used as a therapeutic tool, and other less invasive radiological methods should be used for diagnostics. McCune described the first ERCP in 1968 (McCune et al., 1968), and Kawai performed the first therapeutic ERCP in 1974 by cutting the muscles of the ampulla Vateri (Kawai et al., 1974). In Finland, Juhani Lehtola performed the first ERCP in 1974 at Oulu University Hospital. Since then, equipment and techniques have developed enormously.

Table 1. *Indications for ERCP*

Jaundiced patient with biliary obstruction
Choledochal stones
Periampullary tumour
Tumour obstructing biliary tree
Pancreatitis
Portal biliopathy
Biliary trauma after surgery
Leak
Stricture
Sphincter of Oddi dysfunction
Chronic pancreatitis
Pain
Pancreatic fistula
Persistent pancreatic leak after surgery or trauma
Infected or symptomatic pseudocyst
Primary sclerosing cholangitis (PSC) follow-up
Cholangioscopy
Pancreatoscopy

2.1.1 ERCP INDICATIONS AND THERAPEUTIC TECHNIQUES

ERCP is an invasive procedure with potential and even lethal adverse events. The indication of ERCP should always be compared to potential benefits and risks. Table 1 shows the indications of ERCP. Therapeutic options are endoscopic sphincterotomy (ES) (cutting the muscles of the sphincter of Oddi), removal of stones from biliary or pancreatic ducts, stent placement across strictures or large non-removable stones or fistulas, balloon dilatation of strictures and papilla, ampullectomy of adenomatous neoplasia or superficial cancer of the papilla, and intraluminal stone breaking through cholangio- or pancreatoscopy.

2.1.2 ERC IN THERAPEUTICS OF POSTCHOLECYSTECTOMY BILE DUCT INJURIES (BDI)

2.1.2.1 *BDI after cholecystectomy*

Laparoscopic cholecystectomy is one of the most common surgical procedures worldwide. Before the laparoscopic era, the incidence of BDI after cholecystectomy varied between 0.1 and 0.2% (Gouma, Go, 1994). However, after 1990, when laparoscopy became the first choice in the management of symptomatic biliary stones, the incidence of BDI has been reported to increase by between 0.4 and 1.1%, and it has remained high despite advances in technique and technology (Barkun et al., 1997, Nuzzo et al., 2005). However, a first sign of a decreasing trend in incidence has been reported in a large-scale nationally validated database study in the United States, showing 0.19% incidence in post-cholecystectomy BDIs (Mangieri et al., 2018).

Risk factors leading to BDI can be divided into three groups: 1) patient dependent risk factors: obesity, high age, and surgical adhesions; 2) local risk factors: aberrant anatomy, inflammation, infection, haemorrhage, and altered anatomy due to large biliary stones; 3) operation-related risk factors: surgical skills and equipment (Wu et al., 2010).

BDI can be severe and life-threatening, depending on the site and extension of the injury. Bile leaks are the most common type of BDI, and most frequently, in 60-78% of cases, the site of the leak is found in the cystic duct stump (Kaffes et al., 2005, Sandha et al., 2004), and in 2-26% in the aberrant branch of the right hepatic duct (duct of Luschka) (Spanos et al., 2006). The leak can be more severe and originate in 9-20% of BDI cases from the common hepatic duct, common bile duct, or intrahepatic ducts (Sandha et al., 2004, Kaffes et al., 2005, Spanos et al., 2006). Strictures of the bile duct may occur early after a surgical procedure as a consequence of a direct trauma such as a thermal injury, or partial or total clipping of the duct. It can also develop due to local

conditions such as inflammation, infection, or bile leak. Bile duct stricture may develop, or become obvious, months or even years after surgery. Late stricture is considered to develop as a result of periductal inflammation and fibrosis after bile leaks or ischemia due to a damaged local arterial supply. Patients after T-tube reconstruction of partial or total common bile duct laceration have up to a 50 % possibility of developing a stricture (Wudel et al., 2001).

2.1.2.2 *Diagnosis and symptoms of BDI*

Only 15-25% of BDIs are diagnosed during cholecystectomy (Nordin et al., 2002, Way et al., 2003, Karvonen et al., 2011). If BDI is suspected during cholecystectomy, intraoperative cholangiography should be performed to clarify the anatomy and to determine the site of injury. It is sometimes necessary to convert the operation to an open cholecystectomy to avoid further complications. If a skilled hepatobiliary surgeon is available, primary repair is an option. Otherwise, multiple drain placement and a patient referral to a hepatobiliary surgeon is recommended (Perera et al., 2011).

BDI should be suspected in patients with visible bile in drains after surgery, bile emerging from surgical wounds, or in patients who fail to recover and develop abdominal pain after cholecystectomy. Symptoms are often unspecific, including pain, nausea, fever, sepsis, hyperbilirubinemia (from bile peritonitis or biloma), and jaundice (especially in cases of occlusion in the common bile duct). Diagnostic delay increases complications and morbidity. (de Reuver et al., 2007)

Current options for BDI diagnostics are ultra-sound (US), contrast-enhanced computed tomography (CECT), magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), percutaneous transhepatic cholangiography (PTC), and ERCP. US reveals fluid collections and dilated bile ducts. If fluid collection is detected, an US or CT guided percutaneous puncture and aspiration of bile, as well as drain insertion, should be performed to confirm diagnosis (C. M. Lee et al, 2000). CECT is superior to US because it provides more information on intra-abdominal fluid collections, abscesses, bile duct dilatations, and arterial traumas (Mbarushimana et al., 2014). Conventional MRCP has limitations in the evaluation of biliary leaks providing only morphologic information about damage. However, the most accurate diagnosis of biliary leaks is obtained with gadolinium-ethoxybenzyl-diethylenetriamine penta-acetic acid (Gd-BOPTA, Primovist®)-enhanced MRCP, showing the site of the leak in up to 80% of cases (Cieszanowski et al., 2013, Ratcliffe et al., 2014). ERCP visualises the injury and often offers option of simultaneous treatment. If there is a suspicion of total disconnection, PTC is indicated to explore the anatomy of proximal biliary tree, e.g. before biliary reconstruction surgery. If an abdominal drain produces bile after surgery, it can serve as a route for cholangiography (Schipper et al., 1996). Diagnostic laparoscopy or laparotomy is indicated only

if biliary peritonitis does not respond to percutaneous drainage (Nordin et al., 2011).

2.1.2.3 *Classification of BDI*

Several classifications have been developed to describe postoperative BDIs. ***Bismuth*** et al. designed the first classification, which categorises only biliary strictures (Bismuth, Majno, 2001). ***Strasberg*** and colleagues further developed Bismuth's classification to describe not only strictures but also leaks, complete trans-sections, and occlusions (Strasberg et al., 1995). The ***Stewart-Way*** classification with four injury types describes the mechanism of the BDI, as well as its anatomy. It differentiates strictures and resectional injuries, but it does not include cystic stump leaks (Stewart, 2014). Biliary endoscopists often use the ***Amsterdam classification,*** which is very practical for endoscopic purposes and helps to choose the endoscopic treatment. The Amsterdam classification is divided into four classes describing minor and major leaks in the biliary tree, strictures, and occlusion of the main bile duct (Bergman et al., 1996) (Figure 1).

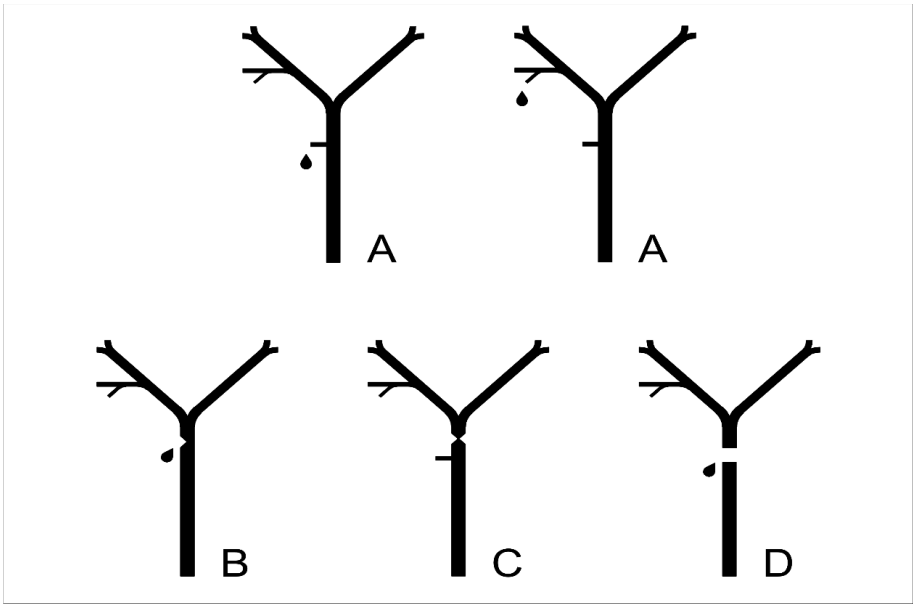

Figure 1 Amsterdam classification A = minor bile duct leaks, B = major bile duct leaks with or without strictures, C = strictures, D = complete trans-sections

2.1.2.4 *Endoscopic treatment options for BDI*

Before the development of ERCP techniques BDIs were treated operatively. Since endoscopic treatment has lower morbidity and mortality compared to surgery, it has become the first method of choice in BDI treatment (Tocchi et al., 2000). In up to 90% of Amsterdam type A, B, and C BDIs after cholecystectomy endoscopic treatment is successful (Karvonen et al., 2011). There is no clear consensus concerning the preferred treatment option due to the scarce prospective research on the subject. Treatment options for leaks are ES with or without stenting, and for strictures ES and stenting. Options for stenting are single or multiple plastic stents, a fully covered self-expandable metal stent (FC-SEMS), bio-degradable stents, or nasobiliary drainage. Usually, in case of BDI, ERC can be performed electively, since timing of the endoscopic treatment has had no any effect on the final outcome (Adler et al., 2017).

The goal of endoscopic treatment in a biliary leak is to reduce the transpapillary pressure gradient. When transpapillary flow is improved by ES, stenting, or nasobiliary drainage, bile extravasation from the leak in the biliary tree will be reduced, allowing the leak to heal (Bjorkman et al., 1995, J. M. Dumonceau et al., 2018). Concomitant bile duct stones impair the bile flow, and they always need to be removed. The bile leak can be graded as low-grade (LG) if the leak of the contrast agent is visible in cholangiography from the distal part of the bile duct only after opacification of the intrahepatic ducts with balloon pressure. The bile leak is graded as high-grade (HG) if the contrast leak is visible before intrahepatic opacification (Sandha et al., 2004).

Biliary postoperative strictures are treated by ERCP with dilatations and stents to re-establish the continuum of the bile duct, correct the pressure gradient, and enable the bile flow to the bowel (J. M. Dumonceau et al., 2018).

Amsterdam type A leaks

The European Society of Gastrointestinal Endoscopy (ESGE) guideline recommends endoscopic placement of plastic stents in the management of minor bile duct leaks to the total transection of the common bile duct or common hepatic duct (J. M. Dumonceau et al., 2018). Results of studies of Amsterdam type A leaks show a high (80-100%) success rate in treatment outcomes, but results of treatment method comparisons are controversial. Some studies have found stenting with or without ES superior to ES alone in all Amsterdam type A leaks (J. M. Marks et al., 1998, Kaffes et al., 2005, Dolay et al., 2010). One study found no difference in these treatment modalities (Mavrogiannis et al., 2006) and another study reported these treatments as equally efficient in LG Amsterdam type A leaks but not in HG Amsterdam type A leaks (Sandha et al., 2004). Plastic stents are the most commonly used stents

in the treatment of type A leaks. The length or size (7 or 10 French) of the stent do not influence the outcome (Katsinelos et al., 2008). If ES is chosen for the treatment, there is usually no need of further endoscopic procedures. Plastic stents always need to be removed in a second endoscopic session, usually after 4-8 weeks in BDIs (J. M. Dumonceau et al., 2018). Biodegradable stents are the most recent innovation in stent development and in post-cholecystectomy BDIs. They are more expensive, but they do not need to be removed (Siiki et al., 2018).

Amsterdam type B injuries

Major biliary leaks with or without strictures are treated with stents to obtain an appropriate flow through the papilla and to bridge major lacerations of common or hepatic bile ducts (Bergman et al., 1996). Quite often, a simple plastic stent is not efficient enough for a high-output bile leak from the common or hepatic bile ducts, and filling the bile duct lumen with multiple plastic stents or FC-SEMSs is therefore a better treatment option (Luigiano et al., 2013, Canena et al., 2015). Plastic stents need to be removed or changed at scheduled intervals of 3 months, and metallic stents at intervals of 6-12 months (Dumonceau et al., 2018).

Amsterdam type C strictures

Biliary strictures are treated successfully with balloon dilatations with multiple plastic stents or FC-SEMS. Usually, after 6-12 months of multiple plastic stents or FC-SEMS, a stricture is resolved (Costamagna et al., 2010a, Luigiano et al., 2013, J. M. Dumonceau et al., 2018). In difficult strictures, when passing the stricture with guidewire and instruments is impossible, the rendezvous technique with an interventional radiologist may be helpful (Gronroos, 2007).

Amsterdam type D injuries

Surgery is nearly always the primary treatment option for almost all type D injuries (Karvonen et al., 2007). In case of a total trans-section, it may be possible to re-establish the continuity of the injured common bile duct with the PTC-ERC combined rendezvous technique (Fiocca et al., 2011, Donatelli et al., 2014).

Operative treatment of biliary injury

Most severe BDIs (Amsterdam type C and D) with disruption of the continuity of the main bile duct are treated operatively. The optimal surgical repair method depends on the type of BDI, the duration of the biliary obstruction, the degree of liver damage, the history of previous biliary repair surgery, and the patient's general condition. Surgical repair options are an end-to-end choledocostomy, Roux-en-Y anastomosis between bile duct and jejunum, liver resections, and, in the most severe cases, hepatectomy and liver transplantation (Parrilla et al., 2014, Halbert et al., 2016). Referral of BDI patients to a hepatobiliary surgeon is highly recommended to avoid complications (Perera et al., 2011).

2.1.2.5 ***Outcome of the treatment of BDI***

The success rate of the endoscopic treatment of Amsterdam type A leaks has increased by up to 100% (Kaffes et al., 2005, Mavrogiannis et al., 2006). The treatment of an Amsterdam type B leak has traditionally been partly operative, and long-term results of endoscopic treatment are rare and come from small series. The success rate of Amsterdam type B leak has been reported to be 71%, or 3.3 times worse than when treating type A leaks (Bergman et al., 1996, Tewani et al., 2013). Treatment of BDI has changed in the direction of endoscopy and improved in the last 10 years with the availability of FC-SEMSs. Furthermore, results of the endoscopic treatment of Amsterdam type C BDI are scarce. One study of patients with a type C injury between 1991 and 2006 reports a primary success rate in stricture treatment of 78%, reaching 91% after further ERCs (Vitale et al., 2008). Endoscopic treatment of benign biliary strictures of aetiologies other than BDI after cholecystectomy has been reported to have success rates of 85-94%. Recurrent stricture after endoscopic treatment occurs usually within 1 to 2 years but is treatable by ERC and stenting (Deviere et al., 2014, Tringali et al., 2016, Costamagna et al., 2010b). Late complications in stent treatment are stent clogging, with or without cholangitis and jaundice, or stent migration. However, these mild complications resolve quickly after stent exchange (Rauws, Gouma, 2004). Recurrent attacks of cholangitis have been reported in 3% of cases during a 5-year follow-up (Boerma et al., 2001).

The incidence of late postoperative stricture after hepaticojejunostomy is 17-30%, and it usually appears within the first 2 years (Schmidt et al., 2005, Stilling et al., 2015). Risks in stricture development are multiple attempts of anastomosis repair, postoperative bile fistula, anastomosis of a non-dilated bile duct, T-drain site after surgery, associated vascular injury, and a highly situated injury in the biliary tree. If stricture leads to secondary biliary

cirrhosis, the patient may be a candidate for a liver transplant. (Schmidt et al., 2005, Gad et al., 2018)

2.1.3 ERCP ADVERSE EVENTS

The most common complications after ERCP, with a total prevalence of 4-11%, are post-ERCP pancreatitis (PEP), bleeding during and after ERCP, cholangitis, and perforation (Cotton et al., 1991, Cotton et al., 2009, Vandervoort et al., 2002).

Post-ERCP bleeding most commonly originates from biliary and/or pancreatic ES, with a rate of 0.3% to 2% (Freeman et al., 1996, Cotton et al., 2009). Coagulopathy, active cholangitis, anticoagulant therapy within 3 days after ERCP, endoscopist case volume < 1 per week, and occurrence of bleeding during the procedure are risks for bleeding (Freeman et al., 1996). Balloon dilatation is a safer procedure than ES for patients with risks of bleeding

Post-ERCP cholangitis occurs in 0.5% to 1.4% of ERCP cases (Cotton et al., 2008, Ismail et al., 2012, Andriulli et al., 2007). Risk factors for cholangitis are combined percutaneous-endoscopic procedures, stenting of a malignant stricture, failed biliary access of drainage, incomplete stone removal, and previous liver transplantation (Freeman et al., 1996, Cotton et al., 2008).

Post-ERCP perforation occurs in 0.08% to 0.6% ERCPs, with an associated 8% to 23% mortality rate (Cotton et al., 2009). Duodenal perforation by the endoscope, extramural guidewire passages, extension of the ES incision over the intramural segment of the bile or pancreatic duct, and stent migration may cause perforation and peritonitis.

PEP is the most common and feared post-ERCP complication resulting from complex and multifactorial reactions in the pancreatic gland after pancreatic duct imaging and/or instrumentation (Cotton et al., 2009).

2.1.4 RISK FACTORS AND PROPHYLAXIS OF PEP

Prevalence of PEP varies in studies, depending on patient selection and the endoscopist's competence, between 2% and 9%, and the prevalence of severe PEP between 0.3% and 0.6% (Freeman et al., 2004, Andriulli et al., 2007). Causes of PEP can be divided into procedure-related and patient-related mechanisms (Table 2). Procedure-related triggering factors are trauma (e.g. guide wire manipulation or ES, causing oedema and spasm leading to pancreatic duct obstruction), increased pancreatic duct pressure (due to contrast injection), and the inoculation of intestinal bacteria in the pancreatic duct (Rustagi et al., 2015). Patients with more than one risk factor have a significantly higher risk of PEP than those with a single risk factor (Freeman et al., 2001).

Table 2 *Risk factors for PEP according to ESGE (J. Dumonceau et al., 2014) and literature*

	Odds ratios (95% confidence intervals)	Incidence of PEP in patients with vs without risk factor
Patient-related risk factors		
Definite risk factors		
Female gender	3.5 (1.1-10.6)	4.0% vs 2.1%
Previous acute pancreatitis	2.46 (1.9-3.1)	6.7% vs 3.8%
Suspected sphincter of Oddi dysfunction	1.91 (1.4-2.7)	8.6% vs 2.5%
Likely risk factors		
Previous PEP	8.7 (3.2-23.9)	30% vs 3.5%
Young age	Range 1.1-2.9	6.2% vs 2.6%
Non-dilated extrahepatic bile ducts		3.8% vs 2.3%
Absence of chronic pancreatitis	1.9 (1.0-3.5)	4.0% vs 3.1%
Normal serum bilirubin	1.9 (1.2-2.9)	4.1% vs 1.4%
Procedure-related risk factors		
Definite risk factors		
Cannulation duration >10 minutes	1.8 (1.1-2.7)	10.8% vs3.8%
Pancreatic guidewire passages >1	2.8 (1.8-4.3)	2.9% vs 9.5%
Pancreatic injection	2.2 (1.6-3.0)	3.3% vs 1.7%
Likely risk factors		
Pre-cut sphincterotomy	2.3 (1.4-3.7)	5.3% vs 3.1%
Pancreatic sphincterotomy	3.1 (1.6-5.8)	2.6% vs 2.3%
Large-balloon sphincter dilatation	4.51 (1.5-13.5)	9.3% vs 2.6%
Failure to clear bile duct stones	3.4 (1.3-9.1)	1.7% vs 1.6%
Intra-ductal ultrasound	2.41 (1.3-4.4)	8.4% vs 2.8%

To prevent PEP, it is crucial to avoid unnecessary ERCPs by choosing correct indications and patients. The potential benefit vs the risk needs to be considered carefully. Some, often necessary, ERCP techniques increase the PEP rate, but a few technical strategies, such as guide wire cannulation, early pre-cut ES, pancreatic stent placement, and the double guide wire cannulation technique, may reduce attempts at cannulation and decrease the risk of PEP (J. Dumonceau et al., 2014, Gronroos et al., 2011). Clinical conditions, such as existing biliary ES, chronic pancreatitis, and malignancy in the head of the pancreas, are considered to protect against PEP (Loperfido et al., 1998, Freeman et al., 2001, Elmunzer, 2017).

2.1.4.1 *Pharmacological prevention of PEP*

Although patient- and procedure-related risk factors are well known and considered carefully, the PEP rate has not improved. Many pharmacological agents have been investigated in PEP prevention, but results have been disappointing. Tested agents such as glucagon (Silvis et al., 1975), calcitonin (Odes et al., 1977), nifedipine (Sand et al., 1993), octreotide (Arcidiacono et al., 1994), and corticosteroids (Dumot et al., 1998, Zheng et al., 2008, Kubiliun et al., 2015) have been ineffective in PEP prevention. However, peri-procedural aggressive intravenous hydration with ***lactated ringer's solution*** reduced 53% of the PEP rate (Zhang et al., 2017) by enhancing pancreatic perfusion and tissue oxygenation, and optimising the pH level (Buxbaum et al., 2014). ***Somatostatin*** has been shown to have some effect in PEP prevention, but it is not recommended except in some selected cases (Wang et al., 2018). ***Nitroglycerin,*** as a smooth muscle relaxant, may lower sphincter of Oddi pressure and enhance pancreatic blood flow (Staritz et al., 1985). Some randomised clinical trials (RCTs) of nitroglycerin show reduced incidence of PEP, but the results are controversial and need more exploration (Kubiliun et al., 2015). ***Nafamostat*** is a protease inhibitor that inhibits trypsin and has been shown to reduce PEP by up to 60% (Yuhara et al., 2014). However, this is expensive and needs prolonged intravenous infusion (7-25 h) making its use problematic. In addition, the preventive effects of nafamostat on PEP in high risk cases are lacking (Kubiliun et al., 2015). Currently ***non-steroidal anti-inflammatory drugs (NSAIDs)*** appear to be the most efficient and studied agents for PEP prevention (Yu et al., 2018).

2.1.4.2 *NSAIDs in prevention of PEP*

It is thought that PEP develops due to a pro-inflammatory cascade originating from a pancreatic acinar cell injury. Phospholipase A2 is one of the key modulators on this cascade. NSAIDs are potent phospholipase A2 inhibitors as well as they also inhibit prostaglandin synthesis and neutrophil/endothelial cell attachment (Makela et al., 1997, Davies et al., 1997). Many studies have shown this inhibitory mechanism and the use of rectal NSAIDs (indomethacin and diclofenac) to reduce PEP in 40-70% of cases. Most of these studies were conducted within high risk patients (Elmunzer et al., 2012, Khoshbaten et al., 2008, Otsuka et al., 2012, Sotoudehmanesh et al., 2007).

Indomethacin has been superior to diclofenac in PEP prevention in separate RCTs, but no comparative study on these two agents has been undertaken. Only rectal NSAIDs have been effective in PEP prevention, and NSAIDs administered through other routes (oral, intramuscular, or intravenous) have been useless (Cheon et al., 2007, Park et al., 2015, de Quadros Onofrio et al., 2017). Rectal administration provides maximal drug

bioavailability, faster absorption and rapid concentration, suppressing the inflammatory responses of PEP. The oral route has a different metabolism from the rectal route due to gastro-hepatic circulation, which explains NSAIDs' ineffectiveness in PEP prevention. The reason intravenously or intramuscularly administered NSAIDs are powerless in PEP prevention is unclear (Lyu et al., 2018). ESGE guidelines suggest routine use of rectal NSAIDs for all ERCP patients without contraindications. This recommendation is based on 6 meta-analyses (J. Dumonceau et al., 2014).

2.1.4.3 *Pancreatic stent placement in prevention of PEP*

ERCP-induced papillary oedema may increase pressure within the pancreatic duct. Pancreatic stent placement is thought to reduce this pressure and decrease the risk of PEP development (Tarnasky et al., 1998, Fogel et al., 2002). Controversially, the stent placement may enhance the risk of PEP, especially if stent placement is attempted but remains unsuccessful (Freeman et al., 2004). A pancreatic stent is therefore recommended only in cases of difficult cannulation in patients with a high risk of PEP (J. Dumonceau et al., 2014).

2.2 ACUTE PANCREATITIS (AP)

2.2.1 EPIDEMIOLOGY AND AETIOLOGY OF AP

Acute pancreatitis (AP) is one of the most common gastrointestinal disorder globally. In Finland, the incidence of AP has been the highest in Europe, 73-102/100,000 (Jaakkola et al., 1993, Pelli et al., 2009). During recent decades, the trend of AP worldwide has been increasing (Roberts et al., 2017, Krishna et al., 2017), but a recently published survey from the United States shows stabilisation, and even a decrease for the first time, in the incidence of AP (Sellers et al., 2018). Although AP related mortality has decreased to 0.79% due to better diagnostics for the disease (Krishna et al., 2017), in severe AP (SAP) it is still as high as 20-42% (Harrison et al., 2007, Pavlidis et al., 2013, Karjula et al., 2017). APs induced by different aetiological factors, e.g. biliary stones, alcohol, and hypertriglyseridemia, do not differ in mortality rates (Andersen et al., 2008, Goyal et al., 2016).

Table 3 *Aetiology of AP*

Toxic:	Genetic mutation in:
Alcohol	CFTR
Medicines	PRSS1
Drugs	PRSS2
Tobacco	CTRC
Viruses	CASR
Parasites	SPINK1
Obstructive:	Traumatic:
Cholelithiasis	ERCP
Papillary tumours	Penetrating and blunt injuries
Duodenal diseases	Surgery
Pancreas divisum	Pancreatic biopsy
Sphincter Oddi dysfunction	
Endocrine/metabolic:	Other:
Hypertriglyseridemia	Idiopathic
Hypercalcemia	Autoimmune diseases

There are regional variations in the aetiology of AP, related to alcohol consumption habits and the prevalence of gallstones. In southern Europe, gallstones are the dominant reason for AP (Roberts et al., 2017), whereas in Finland, alcohol causes about 70% and gallstones 20% of AP cases (Halonen et al., 2000, Jaakkola et al., 1993, Karjula et al., 2017). Alcohol-related AP is more common in men than in women, but with similar consumption habits

this difference disappears (Lankisch et al., 2002). In addition to ethanol and gallstones, AP has a variety of other aetiologic factors, and 10% of AP cases remain idiopathic (Table 3). Some of the idiopathic APs are explained by under 3 mm gallstones that are radiologically poorly visible in papilla Vateri or gallstones that have already passed through the papilla, inducing AP (Raty et al., 2015). Old age, obesity, and a meat-rich diet are factors that have been associated with an increased risk of AP (Yadav et al., 2013, Dugum et al., 2018). Alcohol-induced AP patients are prone to hospital re-admissions compared with patients with other aetiologies (44% vs 22%, respectively) (Karjula et al., 2017).

2.2.2 PATHOGENESIS OF AP

The pathogenesis of AP has been widely researched, but the exact mechanism remains unresolved. Pancreatic exocrine glands consist of acinus formed of digestive enzymes secreting acinar cells. Pancreatic ducts formed of ductal cells secrete 2.5 l/day of alkaline $HCO3^-$-rich fluid, which neutralises acidic content secreted by acinar cells. Ducts provides a structural framework for the pancreas. They also convey digestive enzymes from acinar cells (M. G. Lee et al., 2012). Pancreatic secretion is regulated by hormonal and neural stimulation, mediated by secretin and cholecystokinin (Chandra et al., 2012). Stellate cells surrounding acinar and ductal cells are thought to play a key role in the repair and recovery of the gland (Apte et al., 2012). Multiple triggering factors initiate pancreatic inflammation in acinar, ductal, and stellate cells.

Alcohol directly affects the pancreatic acinar, ductal, and stellate cells, increasing the cytosolic calcium concentration, which triggers the inflammatory process of AP. Ethanol is metabolised via an oxidative or a non-oxidative pathway, producing toxic metabolites (acetaldehyde and fatty acid ethyl esters) that tend to injure the pancreas (Haber et al., 1998, Gukovskaya et al., 2002, Apte et al., 2010). In acinar cells, alcohol metabolism causes increased synthesis of digestive and lysosomal enzymes, enhancing their potential contact and premature activation (Norton et al., 1998). Alcohol also induces bacterial leak from the bowel into the pancreas, and this has been shown to be one of the triggering factors for AP (Vonlaufen et al., 2007). Although the risk of AP is known to increase with heavy alcohol use, only some heavy drinkers develop symptomatic AP (Durbec et al., 1978, Steinberg et al,. 1994). Additional factors to alcohol are needed to initiate AP, but this mechanism remains unclear**.** ***Bile acid*** reflux has also been shown to have direct effects on ductal, acinar and stellate cells (Hegyi et al., 2015, Kim et al., 2002, Ferdek et al., 2016). Both bile acids and ethanol in high concentrations inhibit ductal $HCO3^-$ secretion and increase cytosolic calcium concentration. A decreased extracellular pH enhances both trypsinogen auto-activation in the pancreatic ducts and injury in acinar cells (Maleth et al., 2011). Biliary stones, tissue swelling after ERCP, a tumour, or pancreatic stones can cause

pancreatic ***duct obstruction,*** leading to an upstream blockage of pancreatic secretion and the inhibition of the exocytosis of digestive enzymes initiating AP (J. Dumonceau et al., 2014, Mujica et al., 2000).

Regardless of the aetiology of AP, the inflammation reaction is quite similar. The progression of the disease has three steps: local pancreatic inflammation seen in mild AP; general systemic inflammatory response syndrome (SIRS); and multiple organ failure in SAP (Bhatia et al., 2005).

2.2.2.1 *Calcium signalling*

Short-lasting physiological local calcium signals control normal fluid and enzyme secretion in acinar cells. Bile acids, alcohol metabolites, and low extracellular pH levels increase calcium release within acinar cells, leading to persistent high cytosolic calcium concentrations (Petersen et al., 2006, Petersen et al., 2009, Reed et al., 2011). Excessive intracellular calcium triggers trypsinogen activation within the acinar cells. It also leads to oxidative stress, resulting in necrosis (Mukherjee et al., 2008). A high calcium level also activates nuclear factor κB (NF-κB), which plays a key role in developing acinar cell damage and SIRS (Gerasimenko et al., 2014). Recently, calcium signalling has been shown also to occur in stellate cells. Dying acinar cells release trypsin, which increases calcium concentration in stellate cells, leading to the production of nitric oxide. It in turn diffuses to the acinar cells, promoting the necrotic process and creating a vicious circle of necrotic acinar cell death (Gryshchenko et al., 2018).

2.2.2.2 *Nuclear factor κB*

NF-κB is a signalling molecule responsible for regulating the production of a variety of mediators involved in immunity and inflammation: chemokines, cytokines, and adhesion molecules recruit inflammatory cells to the site of inflammation (Sen et al., 1986, Baldwin, 1996). In the early stage of AP, intra-acinar activation of NF-κB directly triggers the inflammatory pathway, causing SIRS, and worsens the severity of AP. Anti-inflammatory agents have been shown to be effective inhibitors of pancreatic NF-κB activation in experimental AP (Rakonczay et al., 2008, H. Huang et al., 2013). Although acinar NF-κB and trypsinogen activation occur with similar time and are both induced by increased intracellular calcium concentration, they are independent events (Dawra et al., 2011).

2.2.2.3 *Trypsinogens*

The pancreas secretes digestive enzymes as inactive precursors in acinar cells. Trypsinogens are the most important digestive proenzymes of the proteins in pancreatic juice. In normal conditions, the acinar cell is protected from enzymatic damage by secreting digestive enzymes as inactive forms (zymogens), storing the enzymes inside membrane-bound compartments, synthesizing and releasing trypsin inhibitors simultaneously with the zymogens, maintaining low intracellular calcium concentrations favouring trypsin degradation, and maintaining the acidic pH inside the zymogen granules, which inhibits trypsin activity (Gorelick et al., 2009).

Pancreatic secretory trypsin inhibitor (PSTI), also called Kazal-type trypsin inhibitor (SPINK1) after the only gene encoding it, was first identified in urine in ovarian cancer patients and called tumour-associated trypsin inhibitor (TATI) (Huhtala et al., 1982, Stenman et al., 1982). $SPINK_1$ prevents premature activation of trypsinogens within the acinar cells in normal conditions (Pubols et al., 1974). In normal conditions, only a minority of trypsinogens leak into the circulation, where the trypsin inhibitors (alpha-1-antitrypsin and alpha-2-macroglogulin) deactivate active trypsin (Borgstrom et al., 1978a).

As early as1896, Hans Chiari proposed that premature activation of trypsinogens in acinar cells led to AP. This theory has since been frequently studied in experimental and clinical pancreatitis. Although many details have been solved, the exact mechanism of AP pathogenesis has remained obscure. Intra-acinar hyper-concentration of calcium triggers intracellular premature activation of trypsinogen (A. U. Shah et al., 2009) and cathepsin B, a lysosomal hydrolase, serving as a mediator in this process (Van Acker et al., 2002). If the activation of trypsin exceeds the intra-acinar trypsin inhibition system, it results in the release of pancreatic enzymes, eventually causing acinar cell damage and death. This process is responsible for half of pancreatic necrosis. Acinar cell damage leads to release of damage-associated molecular patterns (DAMPs), an overdose of which in turn activates cytokine production and inflammation cell recruitment (Martinon et al., 2009). Trypsin has also been found to be a triggering factor for stellate cells to join AP pathogenesis (Gryshchenko et al., 2018).

Human pancreatic juice contains three trypsinogens with different isoelectric points. The first trypsinogens found were cationic trypsinogen-1 (T-1) and anionic trypsinogen-2 (T-2) (Figarella et al., 1969). A third minor trypsinogen (mesotrypsinogen, trypsinogen-3 [T-3]) with intermediate electrophoretic mobility was found 10 years later (Rinderknecht et al., 1984). Trypsinogens also appear in extrapancreatic tissues: vascular endothelial cells; skin; the oesophagus; the stomach; the small intestine; the lungs; the kidneys; the liver; the bile ducts; the spleen; and neuronal cells (Koshikawa et al., 1998).

T-1 and 2 represent about 19% of the proteins in pancreatic juice. Concentrations of T-1 are twice those of T-2 (Figarella et al., 1969). The average concentration of T-1 and T-2 is 15-26 μg/l (Borgstrom et al., 1976, Florholmen et al., 1984) and 5.5 μg/l (Largman et al., 1978) respectively. ERCP-induced pancreatitis studies have shown that serum T-1 and T-2 levels increase 10-fold as early as 2 h after ERCP, and the levels reach their peak in 6 h. T-1 concentration decreases within 48 hours of the onset of the disease, whereas T-2 concentrations remain elevated for several days (Kemppainen, et al., 2005). In alcohol-induced AP, T-2 is more elevated than T-1, whereas in biliary-induced AP, the ratio is contrary. This has been shown to be helpful in discriminating between biliary- and alcohol-induced pancreatitis (Andersen et al., 2001).

T-3 is the minor isoenzyme, occurring in low concentrations in pancreatic juice, where it represents only 0.5% of the proteins (Rinderknecht et al., 1984). SPINK1 cannot inhibit T-3 as it can T-1 and T-2, but T-3 can digest SPINK1 instead (Szmola et al., 2003). As well as T-1 and -2, T-3 concentration rises in AP patients. In healthy individuals the concentration of T-3 is 1-4.4 μg/l, whereas in mild AP the median concentration is 9.5 μg/l, and in severe 15 μg/l. However, T-3 has not been found effective in predicting the severity of AP (Oiva et al., 2011).

A complex between trypsin-2 and α_1-antitrypsin (Trypsin-2-AAT) is formed when α_1-antritypsin, one of the major human circulating trypsin inhibitors, inactivates trypsin-2 which escapes into the circulation. Normally, enterokinase inactivates trypsinogens in the duodenum (Borgstrom, Ohlsson, 1978b). Trypsin-2-AAT serum level increases more slowly than T-1 or T-2, and in severe AP cases it increases for up to 5 days (Kemppainen et al., 1997, Lempinen et al., 2005). The complex has been shown to be more accurate than T-2, C-reactive protein (CRP), or amylase in the diagnosis of AP and in the assessment of the severity of AP (Hedstrom et al., 1996, Lempinen et al., 2003). The ratios between T-1 and trypsin-2-AAT may help in recognising alcohol-induced AP (Andersen et al., 2001).

SPINK1 is synthesised in pancreatic acinar cells, and it is secreted with trypsinogens and other digestive enzymes (Rinderknecht, 1986). It represents 0.1-0.8% of the total protein of pancreatic juice, and its main function is to prevent and defend against premature activation of trypsinogens in 1:1 molar ratio (Pubols et al., 1974, Rinderknecht, 1986). Mutation in the SPINK1 gene is more common in patients with AP compared to the general population (Whitcomb, 1999).

SPINK1 is known to behave as an acute phase reactant, and in addition to pancreatic origin, it has also been detected in the liver, brain, spleen, lung, kidney, stomach, small intestine, duodenum, colon, appendix, gallbladder, urinary tract, ovary, prostate, and breast (Itkonen et al., 2014). SPINK1 as

TATI, is a useful biomarker for diagnostic and prognostic purposes, particularly in ovarian cancer, but in many other cancers too (Huhtala et al., 1982, Stenman, 2002). Serum concentrations of SPINK1 in the normal healthy population are 3-16 µg/l. Due to its small molecular size, its half-life in the circulation is only 6-8 mins (Eddeland et al., 1978b, Marks et al., 1983). In AP, inflammation and necrosis leads to a leakage of SPINK1 into the circulation and urine, increasing SPINK1 concentrations up to 100-fold (Eddeland et al., 1978a, Hedstrom et al., 1996a).

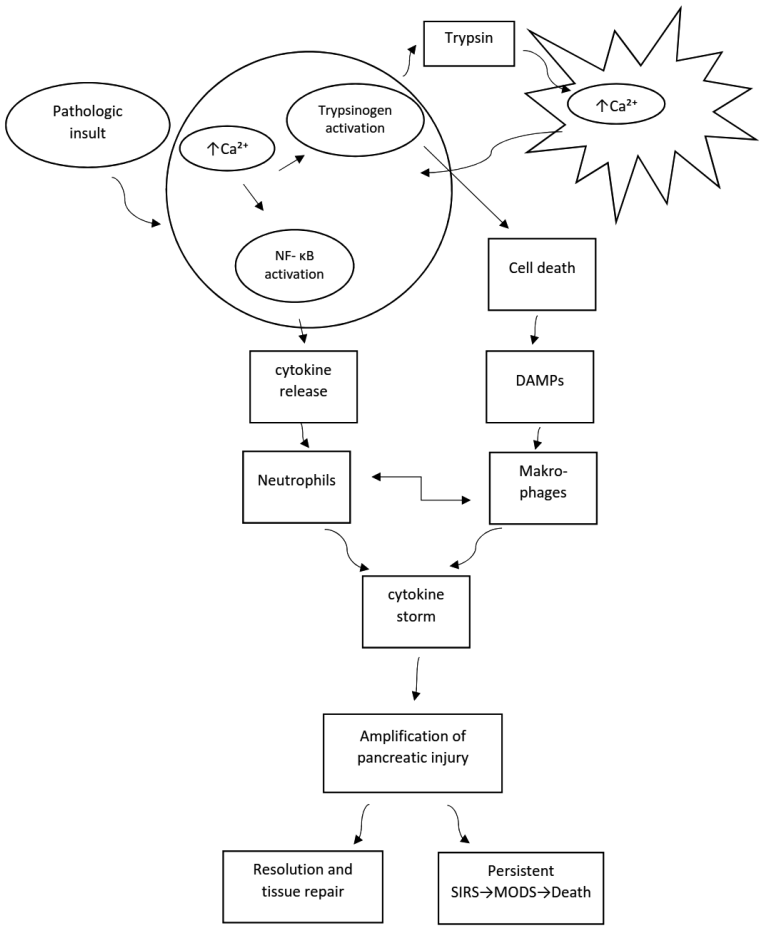

Figure 1 *Inflammation in AP arising in acinar and stellate cells. Acinar cells respond to pathologic insult, activating NF-κB and leading to the production of cytokines and other mediators that initiate the inflammatory response. Trypsinogen activation causes cell death and the release of damage-associated molecular patterns (DAMPs) from damaged cells, which activates inflammasomes. The inflammatory mediators recruit neutrophils, macrophages, and T-cells into the pancreas, leading to a cytokine storm and a systemic inflammation reaction (SIRS), and multiple organ dysfunction (MODS). Trypsin also causes an increase in calcium levels in stellate cells, triggering the production of nitric oxide, enhancing further acinar cell damage.*

2.2.2.4 *Inflammation of AP*

Inflammation is a protective physiological immune system response to tissue injury, which aims to eliminate damaged cells and initiate tissue repair. Immune responses work as part of a network of cellular and humoral responses. Secreted mediators, cytokines and chemokines, coordinate the inflammatory cells (monocytes, macrophages, polymorphonuclear leucocytes, eosinophils, basophils, mast cells, T- and B-cells, and natural killer cells). (Gukovsky et al., 2013)

Pancreatic enzymes cause local destruction in acinar cells, and injured acinar cells activate numerous inflammatory pathways. Activated NF-κB produces cytokines, causing a cytokine storm, which in turn recruit neutrophils, macrophages, monocytes and lymphocytes to the pancreas (Baldwin, 1996). Activated trypsin causes cell death and damage, and damaged necrotic cells release DAMPs and other molecules, which also activates multiple inflammatory pathways, leading to the production and release of inflammatory cytokines, interferons, chemokines and cell adhesion molecules to the inflammation sites and increasing the inflammation and the severity of AP (Hoque et al., 2012)(Figure 1).

Several studies have shown an association between cytokines and chemokines with remote OD and suggest that systemic complications during AP result from an uncontrolled activation of an inflammatory cascade (Aoun et al., 2009, Ueda et al., 1996, Espinosa et al., 2011, Muller et al., 2000, Nieminen et al., 2014). An excessive stimulation of the inflammatory cascade causes early systemic complications and SIRS, within the first week of AP (Table 4). In the early course of AP, infections are rare, and necrosis is sterile. However, during SIRS, there is a higher risk of complicated AP and organ failure (Mayer et al., 2000, Bone, 1996a, Rangel-Frausto et al., 1995). Persistent SIRS, lasting over a week, leads to mixed inflammatory response syndrome (MARS) and further to the compensatory anti-inflammatory response syndrome (CARS). At this point of the inflammation process, pro-inflammatory and anti-inflammatory mediators battle in a micro-environment, often resulting in an unbalance and the finding of both pro- and anti-inflammatory mediators in the circulation (MARS) (Phillip et al., 2014). If the mediators achieve balance, homeostasis is restored. Without balance, eventually either a massive SIRS or CARS will ensue. During CARS, the immune system is downregulated, and general infections, as well as infections of pancreatic or peripancreatic necrotic tissues, are more frequent. During SIRS, a predomination of cardiovascular problems, apoptosis, and organ dysfunction is apparent. (Bone, 1996b) (Figure 2)

Figure 2 *Phases of severe pancreatitis. CARS: compensatory response syndrome, SIRS: systemic inflammatory response syndrome*

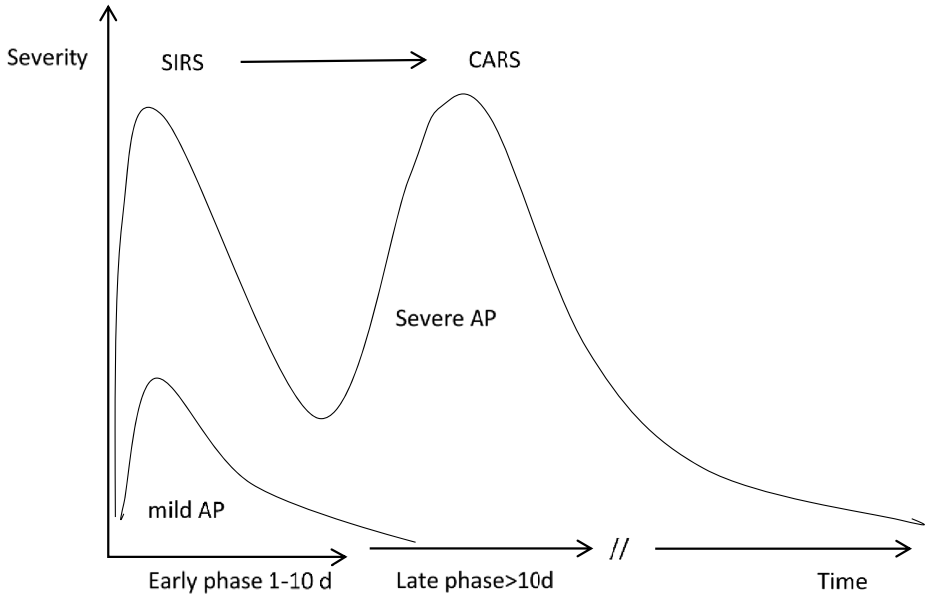

Table 4 *Criteria of systemic inflammatory response syndrome; two or more present criteria defines SIRS (Bone, 1992)*

Heart rate	>90 beats/min
Core temperature	<36 ºC or > 38 ºC
White blood count	<4 x 10^9 /L or > 12 x 10/L or > 10% immature forms
Respiratory rate	>20/min or PCO2 < 32 mmHg

2.2.2.5 *Complications of AP*

The course of AP may cause local and remote complications. Local complications appear in pancreas parenchyma and surrounding tissue as peripancreatic fluid collections, and pancreatic or peripancreatic necrosis. During AP, the fluid collections and necrosis can develop to pseudocysts and walled-off necrosis (WON) (Sarr et al., 2013). SIRS caused by excessive stimulus of cytokines generates remote complications in renal, respiratory and cardiovascular organ systems. Organ dysfunction (OD) develops in 40% of patients with AP, and in SAP it has been associated with increasing mortality (Buter et al., 2002). According to the Modified Marshall Score (MMS) the presence of organ dysfunction (OD) is identified if the calculated organ failure score is 2 or more and the failure affects one or more of the respiratory, renal and/or cardiovascular organ systems. The criteria for scoring the OD are presented in Table 5 (Marshall et al., 1995).

Table 5 *Modified Marshall Score (Marshall et al., 1995)*

	Score				
Organ system	0	1	2	3	4
Respiratory (PaO2 / FiO2)	> 400	301-400	201-300	101-200	≤ 101
Renal (serum creatinine µmol/l)	≤134	134-169	170-310	311-439	> 439
Cardiovascular (systolic blood pressure, mm Hg)	> 90	< 90, fluid responsive	< 90, not fluid responsive	< 90, pH < 7.3	< 90, pH < 7.2

2.2.3 DIAGNOSIS OF AP

The diagnosis of AP requires two of three diagnostic features: 1) abdominal pain; 2) serum or plasma amylase or lipase concentrations at least three times greater than the normal upper limit; and 3) characteristic findings of AP from abdominal imaging (CECT or MRI, rarely US). Radiological examinations should be reserved for patients whose diagnosis is unclear, who fail to improve clinically within the first 48-72 h, or have possible complications (Banks et al., 2006, Tenner et al., 2013).

2.2.3.1 *Clinical symptoms*

The main symptom of AP is usually severe and persistent epigastric abdominal pain resembling peritonitis. The pain intensity varies without reflecting the severity of the disease. In gallstone-induced AP, the onset of the pain can be sudden and knife-like, and may radiate to the back. In alcohol-induced, hereditary or metabolic AP, the onset may be less abrupt. Additional symptoms are nausea, vomiting, fever, tachycardia, dyspnoea and abdominal distension, as well as hemodynamic instability in severe cases (Whitcomb, 2006, Banks et al., 2006). Most patients arrive at hospital within 12-24 h after the onset of symptoms. Cutaneous manifestations, e.g. Cullens sign in the abdomen and flank areas, produced by tracking of liberated pancreatic enzymes to the subcutaneous spaces, are rarely seen signs that indicate a severe form of and poor prognosis for AP (Meyers et al., 1989, Fox, 1966, Sigmund et al., 1954, Lankisch et al., 2009).

2.2.3.2 *Laboratory tests*

The measurement of amylase and lipase has been central in the diagnosis of AP. Amylase is pancreas- and salivary gland-produced glycoside hydrolase, and it can be found in other tissues in lower concentrations. Amylase increases rapidly in blood within 3-6 h of the onset of AP. Due to its short half-life (10-12 h), amylase decreases rapidly and the kidneys excrete it completely within 3-5 days (Pieper-Bigelow et al., 1990, Shah et al., 2010). Hyperamylasemia occurs in several other conditions besides AP: pancreatic diseases and traumas, burns, salivary diseases, gastrointestinal disorders, hepatitis, cirrhosis, gynaecological disorders, cholecystitis, peritonitis, biliary calculus, chronic alcoholism, renal failure, acidosis, pregnancy, head injuries, multiple osteomas, and aortic dissection (Yegneswaran et al., 2010).

Serum lipase rises rapidly 3-6 h after the onset of AP and peaks within 24 h, remaining in the blood longer and at higher concentrations than amylase. Since lipase is primarily synthesised in the pancreas it is more specific for AP than amylase (Shah et al., 2010). Sometimes lipase can be detected during inflammatory bowel disease, intestinal ischemia, malignancies, fat embolism, oesophagitis, and liver and renal failure (Viljoen et al., 2011). Many recommendations prefer lipase over amylase in the diagnosis of AP. However, a meta-analysis by Cochrane Library found no difference between these tests (Lippi et al. 2012a, Rompianesi et al., 2017).

Specific assays for T-1, T-2, and T-3 have been developed, but the complexity and cost of the tests have hampered their use (Itkonen et al., 1990, Oiva et al., 2011). A rapid dipstick test for urine T-2 (Actim Pancreatitis; Medix Biochemica, Kauniainen, Finland) was developed 20 years ago (Hedstrom et al., 1996). The test is easy and quick to use; after dipping in the fresh urine

sample, the result is detectable within 5 minutes. The dipstick test has a detection line as well as a reference line. Both turn blue if the urine T-2 concentration exceeds 50 μg/l, indicating a positive dipstick test. This has been shown to be a reliable diagnostic test for AP and PEP (Kemppainen et al., 1997, M. Kylanpaa-Back et al., 2000). In a Cochrane Library meta-analysis the T-2 dip stick test performed equally in AP and PEP diagnosis to serum amylase and lipase: 10% of cases were diagnosed as positive incorrectly; 25% of cases were not diagnosed with any of the tested markers (Rompianesi et al., 2017).

The serum concentrations of amylase or lipase do not correlate with the severity of the disease. Instead, CRP, a complete blood count, electrolytes, creatinine, blood glucose, liver transaminases, coagulation status, alkaline phosphatase, and total albumin should be measured and repeated during the clinical course (Lankisch et al., 2015).

2.2.3.3 ***Radiological examinations***

CECT with both intravenous and oral contrast agents is the standard imaging technique for the evaluation of AP and its complications (Balthazar et al., 1990, Balthazar, 2002a). Typically in AP, CETC shows focal or diffuse enlargement of the pancreas, an irregular contour of the margins, an increased density of peri-pancreatic fat planes and a thickening of fascial planes, and the presence of intraperitoneal or retroperitoneal fluid collections (Balthazar, 2002b). A CT scan can confirm the AP diagnosis, but it is rarely necessary on admission to hospital if an AP diagnosis is clear. However, if the patient is unstable and does not improve within 48-72 h, a CT scan should be performed to explore possible complications.

Based on the CECT scan, AP can be classified as interstitial oedematous pancreatitis or necrotic pancreatitis. *Interstitial oedematous* AP is a common finding in mild AP, representing 90-95% of all pancreatitis, and it is characterised by localised or diffuse enlargement of the pancreas (Sarr et al., 2013). *Pancreatic necrosis* lacks enhancement after intravenous contrast administration because of thrombosis of pancreatic microcirculation. It can usually be detected in CECT 96 h (sometimes even 48 h) after the onset of the disease (Isenmann et al., 1993). Necrotising AP may be sterile or infected (Sarr et al., 2013). The pancreatic necrosis findings in CECT can be categorised in three groups: 1) encapsulated organised pancreatic necrosis and necrotic peripancreatic fat; 2) central gland necrosis, resulting in the disruption of the pancreatic duct and persistent collections; 3) extra-pancreatic necrosis without pancreatic necrosis (Bharwani et al., 2011).

MRI is an alternative examination modality if CECT is contraindicated due to a contrast allergy or renal dysfunction. The morphological alterations in MRI in AP are very similar to those in CECT (Lecesne et al., 1999). However, MRI distinguishes necrosis in fluid collection better than CECT (Morgan et al.,

1997, Sarr et al., 2013). MRI is superior to CECT in diagnosing biliary stones in the common bile duct (S. L. Lee et al., 2018).

Transabdominal US is cheap and widely available, but there are limitations to its use. US can reveal an enlarged pancreas as a result of oedema, but the accuracy is poor in obese patients or when intestinal gas is present. A normal finding does not therefore rule out a diagnosis of AP (Bortoff et al., 2000).

2.2.3.4 *Classification*

The severity of AP varies from a mild, sometimes subclinical disease, to severe AP (SAP) with OD and even lethal consequences. Patients with mild AP recover spontaneously within a few days, but patients with SAP may develop life-threatening local and/or systemic complications. The Atlanta symposium created a classification system in 1992, and it has since been the standard classification for the severity of AP (Bradley, 1993). The classification has been revised over the years because of a better understanding of organ dysfunction and pancreatic morphological changes (Revised Atlanta Classification). Currently, AP is classified as a mild, moderately severe or severe disease (Table 6) (Sarr et al., 2013). Severity is assessed with CECT, showing possible local morphologic complications, and MMS revealing the presence of OD.

In mild AP, OD and local systemic complications are absent, patients are usually discharged in early phase of the disease and they do not require pancreatic imaging. In moderately severe AP, transient OD (resolves within 48 h) or local peri-pancreatic collections are present. SAP is characterised by persistent OD, which can affect single or multiple organ systems. Patients with persistent OD usually have one or more local complications. An early onset of OD increases the risk of death to 36-50% (Johnson et al., 2004, Mofidi et al., 2006, Buter et al., 2002).

Table 6 *Revised Atlanta Classification (Sarr, Banks et al. 2013)*

	Organ dysfunction	Local complications	Systemic complications
Mild pancreatitis	-	-	-
Moderately severe pancreatitis	Transient (resolves < 48 h)	+/-	+/-
Severe pancreatitis	Persistent (> 48 h)	+/-	+/-

2.2.3.5 ***Severity assessment***

The course of AP varies, and the development of organ dysfunction is the most important determinant for the outcome of AP. To provide optimal care for those in need, it is crucial to distinguish between patients in developing intensive care requiring SAP. Several scoring systems and laboratory tests have been the interest of studies of the optimal severity assessment in the early phase of the disease.

Clinical scoring

Several clinical scores can be calculated from physiology, laboratory, and occasionally radiological parameters to describe the severity of AP and predict the course of the disease. One of the first scoring systems was the Ranson score, originating in 1974 with high sensitivity (84 %) and specificity (78 %) evaluated 48 h after admission (Ranson et al., 1974). Many additional scoring systems, such as Acute Physiology and Chronic Health Evaluation II (APACHE II), the Bedside Index for Severity in Acute Pancreatitis, the Harmless Acute Pancreatitis Score, the Glasgow-Imrie Criteria, the Pancreatic outcome Prediction, and the Revised Japanese Severity Score, have since been developed, but these scoring systems attempt to differentiate only severe cases, and none has been sufficiently accurate to differentiate between transient and persistent OD (Mounzer et al., 2012). Most of these scoring systems are completed at the earliest 24-48 h after admission and are quite complicated to use, requiring several measurable variables.

Laboratory measures and biomarkers

A variety of laboratory tests has been studied as a predictor for the developing of OD. Routinely used tests such as serum creatinine, haematocrit, and calcium reflect the presence of OD and measure intravascular volume depletion rather than predicting OD development. A rising *creatinine* level unresponsive to fluid administration indicates a risk of SAP, and a creatinine level of ≥ 159 μmol/l 48 h predicts the development of pancreatic necrosis (Lipinski et al., 2013, Muddana et al., 2009, Mofidi et al., 2006). *Hematocrit* ≥ 44% at admission predicts SAP, and hematocrit ≤ 44% has a strongly negative predictive value (Brown et al., 2000). *Hypocalcemia* is caused by cathecolamine-mediated calcium translocation from plasma into tissues (Shahbaz et al., 2011). Low calcium levels on admission to hospital have been shown to predict SAP and indicate persistent OD (Mentula et al., 2005a, Peng et al., 2017).

Inflammatory markers

Major acute phase protein *CRP* is most frequently used in the prediction and evaluation of the severity of AP (Puolakkainen et al., 1987, Mofidi et al., 2009). Inflammatory stimuli trigger CRP production in hepatocytes with a delay of up to 72 h, which impairs its use in the early phase of the disease. CRP is unspecific, and it also reflects other inflammation conditions. However, it is shown that CRP > 200 mg/L 48 h after the onset of symptoms is highly predictive of pancreatic necrosis, and CRP > 150 mg/L 48 h after the onset of symptoms can be associated with SAP (Al-Bahrani et al., 2005, Puolakkainen et al., 1987).

Serum *procalcitonin (PCT)* is a precursor of thyroid hormone calcitonin. In healthy adults, it is undetectable. All tissues have the potential to produce PCT, and it is elevated in patients with sepsis and severe inflammation (Becker et al., 2010). Pancreatic necrosis is associated with inflammation, and detectable PCT reflects the complicated course of AP and the need for radiological examination. PCT is already measurable within hours of symptom onset, and it has been found in significantly elevated concentrations in patients with infected pancreatic necrosis and associated ODs or death (M. L. Kylanpaa-Back et al., 2001b, Rau et al., 2007a).

Soluble urokinase-type plasminogen activator receptor (suPAR) is a systemic inflammation marker which increases in many conditions such as inflammation and infection but also in hypoxemia and ischemia in the early stage. It has been shown to predict the outcome of critical illnesses and the development of SAP and lethal AP (Nikkola et al., 2017, Lipinski et al., 2017). However, as a systemic inflammation marker, suPAR is not specific for AP, which affects its use as a predictive tool.

Pentraxin 3 is also an acute phase protein in which plasma concentrations increase rapidly in inflammatory conditions. Various cells in peripheral tissue produce Pentraxin 3, and this is shown to predict severe sepsis and a fatal outcome in critically ill patients (Uusitalo-Seppala et al., 2013) and in the severe form of AP (Simsek et al., 2018).

Since *cytokines* are elevated in the early course of pancreatitis, they have been studied as predictors of OD. Interleukins such as *IL-6, IL-8, IL-10,* and *hepatocyte growth factor* have been found to best predict severe AP among cytokines (Ueda et al., 1996, Mentula et al., 2005b, Aoun et al., 2009). In patients without OD at admission, IL-6, IL-8, and HGF have been found to predict severe AP (Nieminen et al., 2014, Jain et al., 2018). In some clinics, IL-6 is used to identify patients at risk of developing severe disease (Rau et al., 2007b). In addition, other cytokines e.g. such as *IL-1β, IL-12, IL-15, IL-17, IL-2 receptor, IL-1 receptor antagonist, growth-related oncogene alpha, Macrophage migration factor, and granulocyte macrophage colony stimulating factor* have also been studied and found to predict SAP with varying results.

Among the other inflammatory markers examined for the severity assessment of AP are e.g. *CD73/ecto-5´nucleotidase,* an adenosine-generating enzyme which dampens inflammation (Maksimow et al., 2014), *CD11b,* an adhesion molecule of neutrophils and monocytes (M. L. Kylanpaa-Back et al., 2001a), complement regulator protein *CD59* (Lindstrom et al., 2008), and extracellular matrix degradation endopeptidases *matrix metalloproteinase 8 and 9* (Nukarinen et al., 2016).

Markers of pancreatic injury

The most studied *trypsinogen activation marker* are *T-2* (Sainio et al., 1996a, Hedstrom et al., 1996) and *trypsin-2-AAT,* which have shown marked correlation with complicated AP. They have also been shown to be superior to *T-1* in diagnosing AP (Hedstrom et al., 1996, Hedstrom et al., 2001). *SPINK1* concentration is also known to increase in AP in accordance with its severity, but it has not been studied in the prediction of the development of OD (Kitahara et al., 1980, Ogawa, 1988a, Lempinen et al., 2005). *Trypsin activation peptide TAP* is a peptide released from trypsinogen during the activation of trypsin from trypsinogen in severe AP (Formela et al., 1995). When measured from urine, it is shown to be as useful in the assessment of the severity of AP (Neoptolemos et al., 2000, W. Huang et al., 2013, Yasuda et al., 2019).

Carboxypeptidase B Activation Peptide is a trypsin activation peptide which has been shown to be rapidly released into the circulation and urine after the onset of AP. It has been shown to be useful in the prediction of the severity of AP measured from both blood and urine (Deng et al., 2015).

Cell death markers such as circulating DAMPS have also been investigated in the assessment of SAP. It has been shown that nucleosomes predict SAP also in patients admitted to hospital without OD (Kocsis et al., 2009, Penttila et al., 2016).

Phospholipase A_2 (PLA2) is a lipolytic enzyme which generates inflammatory precursors. It is distributed widely in tissues throughout the body, and it is especially strongly present in pancreatic juice and tissue. It is synthesised in acinar cells as inactive precursors, and during AP, it is released into the circulation. PLA2 has been studied as a marker for AP, and it correlates with the severity of AP (Gronroos et al., 1992, Nevalainen et al., 1993). The PLA2 activity profile resembles that of CRP (Puolakkainen et al., 1987), and in SAP, PLA2 correlates with the presence of SIRS (Hietaranta et al., 1999). However, PLA2 assessment has technical limitations with high costs and a cumbersome technique, and it has not been used in the diagnosis of AP (Lippi et al., 2012a).

2.2.4 TREATMENT OF AP

There is no curative therapy for AP. Early therapy consists of supportive treatment, including adequate fluid resuscitation and pain management. In the later course of the disease, interventions may be need.
Guidelines recommend early ***fluid resuscitation*** in the emergency room to reduce morbidity and mortality (Tenner et al., 2013). However, aggressive fluid therapy may lead to respiratory complications and abdominal compartment syndrome (Mao et al., 2010).
A secondary infection of pancreatic or peripancreatic necrosis is thought to be the result of bacterial translocation from the gut (Wittau et al., 2011). ***Antibiotics*** are indicated only if infections are diagnosed with bacterial cultures (Gaieski et al., 2010, Vege et al., 2018).
Early ***oral feeding*** within 24 hours of onset is recommended (Lankisch et al., 2015). Enteral nutrition maintains the mucosal barrier of the gut and decreases bacterial translocation, reducing infection of pancreatic necrosis and other AP complications, as well as the need for interventions for necrosis and MODS (Vege et al., 2018, Windsor et al., 1998).
A necrotic infected collection unresponsive to conservative treatment is an indication for ***intervention***. A step-up approach with catheter drainage is considered standard care. It is followed by a minimally invasive necrosectomy or an open necrosectomy in rare cases (Working Group IAP/APA Acute Pancreatitis Guidelines, 2013, Tenner et al., 2013).

3 PRESENT INVESTIGATION

3.1 AIMS OF THE STUDY

The goal of this study was to investigate ERC in BDI treatment, the prevention of PEP and the diagnosis of PEP and AP. The specific aims were as follows:

I. To explore whether ES or ES and stenting is superior in Amsterdam A class BDIs.

II. To evaluate if rectal diclofenac has a prophylactic effect in PEP in an endoscopic unit with a low PEP rate.

III. To explore the urine T-2 dipstick test in the early diagnosis of PEP.

IV. To explore whether serum SPINK1, T-1, T-2, T-3, and trypsin-2-AAT can predict SAP.

3.2 MATERIALS AND METHODS

This study was designed and carried out in HUH between 2014 and 2018. All the patients in the study were referred to the abdominal centre between 2004 and 2018. The HUH Ethics Committee approved all the study protocols.

3.2.1 PATIENTS

Studies I and II are retrospective studies, studies III and IV are prospective studies. Patients in studies I-III are patients referred to the HUH Endoscopic Unit for ERCP; patients in study IV are patients referred to the HUH emergency room due to AP.

3.2.1.1 *STUDY I*

This study of the treatment of BDI was designed as a retrospective study which included 99 patients referred to the Helsinki University Hospital Endoscopic Unit between 2004 and 2014 due to a suspected BDI after cholecystectomy. All the patients included in the study demonstrated a bile leak or biliary duct stricture on ERC. The patients' clinical course and the outcome of the treatment were retrieved retrospectively from patient files, and all ERC images were reviewed.

Patients with an Amsterdam type A bile leak were grouped into the ES group if the treatment modality was ES alone, and into the ES with stenting group (EST group) if the treatment modality was ES and stenting. Since the study was retrospective, the treatment modality was chosen by the endoscopist based on personal experience and published information. We compared these groups to clarify whether stenting is superior to ES alone in the treatment of Amsterdam type A bile leaks. The inclusion criteria for the whole cohort was visible BDI in a cholangiogram, and for the ES and EST subgroup, analysis demonstrated Amsterdam type A BDI. Patient characteristics are shown in Table 7.

3.2.1.2 *STUDY II*

This study, evaluating the preventive effects of diclofenac in PEP, was designed as a retrospective observational cohort study of 2,000 ERCP patients referred to the Helsinki University Hospital Endoscopic Unit before and after 2013, when routine diclofenac administration for ERCP patients began. 1,000 patients after November 2013 received 100 mg diclofenac rectally before ERCP (diclofenac group [DG]), and 1,000 patients treated without diclofenac before

November 2013 served as a control group (control group [CG]). We compared these groups and clarified whether there were differences between the groups or subgroups in the incidence of PEP. We reviewed patients' records for the required information. The inclusion criterion was age ≥18. The Exclusion criteria were renal insufficiency, NSAID allergy, elevated plasma amylase, and AP diagnosis prior to ERCP. Patient characteristics are shown in Table 8.

3.2.1.3 ***STUDY III***

This prospective study of a urine T-2 dipstick test in PEP diagnosis consisted of 400 ERC patients referred to the Helsinki University Hospital Endoscopic Unit between 2011 and 2018. Inclusion criteria were age ≥18, native papillae, and no history of chronic or acute pancreatitis prior to ERC. Patient characteristics are shown in Table 9.

3.2.1.4 ***STUDY IV***

This prospective study of prognostic biomarkers in severe AP consisted of 239 patients referred to the Helsinki University Hospital emergency room due to AP between 2005 and 2012. All the included patients had a diagnosis of AP with an onset of the disease less than 72 h previously. Exclusion criteria for this study were an age of less than 18 and chronic pancreatitis. Patients were classified in groups based on the severity of AP according to revised Atlanta criteria: mild, moderately severe, and severe. Patient characteristics are shown in Table 10.

Table 7 *Study I: Patient characteristics (n=99)*

Age, years*	60 (27-93)
Female gender	54 (55)
Type of cholecystectomy	
Laparoscopy	58 (59)
Laparotomy	13 (13)
Conversion from laparoscopy to laparotomy	28 (28)
Elective operation	52 (53)
Acute cholecystitis	45 (45)
Reported surgical problems	
Acute cholecystitis	32 (32)
Chronic cholecystitis	17 (17)
Bleeding	5 (5)
Stone in cystic duct	3 (3)
Wide cystic duct	3 (3)
Abscess	2 (2)
Injuries detected intraoperatively	
Bile leak	3 (3)
Common bile duct injury	5 (5)
Complete transection of common bile duct	1 (1)
Biloma drainage after operation	79 (80)
Days of fistula closure *	4 (0-52)
Hospitalisation days after first ERC*	5 (0-65)

*Data are presented as *median (range) or numbers and (%). ERC = endoscopic retrograde cholangiography*

Table 8 *Study II: Patient and procedure characteristics and comparison between control group (CG) and diclofenac group (DG) (n=2000)*

	CG (n = 1,000)	DG (n = 1,000)	*P*
Age, (years)*	63 (18-100)	64 (18-97)	0.358
Female	421 (42)	430 (43)	0.684
ASA grade			
1	31 (3)	39 (4)	
2	244 (24)	215 (22)	
3	536 (54)	589 (59)	
4	189 (19)	157 (16)	0.697
BMI (kg/m2) *	24.8 (13-56)	24.8 (12-56)	0.897
Native papilla	535 (54)	523 (52)	0.624
History of AP	137 (14)	142 (14)	0.747
History of PEP	16 (2)	6 (1)	0.032
Biliary stone	286 (29)	293 (29)	0.762
Biliary stricture	399 (40)	412 (41)	0.566
PSC	4 (0)	8 (1)	0.247
Bile duct injury	38 (4)	20 (2)	0.016
Liver transplantation	36 (4)	14 (1)	0.002
Pseudocysts	99 (10)	84 (8)	0.239
Chronic pancreatitis	216 (22)	209 (21)	0.702
Biliary stent	386 (39)	381 (38)	0.483
Biliary sphincterotomy	500 (50)	468 (47)	0.133
Biliary stone removal	218 (22)	240 (24)	0.124
Pre-cut	15 (2)	18 (2)	0.593
Pancreatic sphincterotomy	192 (19)	158 (16)	0.045
Pancreatic stent	239 (24)	258 (26)	0.313
Pancreatic brush cytology	32 (3)	41 (4)	0.340
Pseudocyst drainage	18 (2)	17 (2)	0.860
Papillectomy	12 (1)	12 (1)	1.000
Native papilla			
Difficult biliary cannulation	168 (17)	160 (16)	0.629
Cannulation time (s)	105 (0-3720)	91 (0-3030)	0.421
Pancreatic opacification	332 (33)	347 (35)	0.479
Duration of ERCP (min) *	21 (3-153)	21 (2-148)	0.005
Hospital days after ERCP*	1 (0-106)	1 (0-87)	0.015

*Data are presented as *median (range) or numbers and (%). ASA = American Society of Anesthesiology; BMI = body mass index; ² ERCP grade according to Cotton classification (Cotton et al., 2011); PSC = primary sclerosing cholangitis.*

Table 9 *Study III: Patient Characteristics (n=400)*

Age, years*	71 (18 - 100)
Female gender	228 (56.9)
ERCP Indications:	
Biliary duct stones	275 (69)
Malignant biliary stricture	118 (30)
Sphincter of Oddi dysfunction	1 (0.3)
Leak after cholecystectomy	2(0.5)
Other	4 (1)
Comorbidities:	
Diabetes	77 (19)
Cardiovascular disease	251 (63)
Pulmonary disease	43 (11)
Elevated pre-ERCP p-bilirubin	302 (76)
Post- ERCP hyperamylasemia ≥ 3 x URL	50 (13)

*Data presented as *median (range) or number of patients (%). URL upper reference limit*

Table 10 *Study IV: Patient Characteristics (n=239)*

Age, years*	**49 (19-86)**	**49 (19-91)**	**48 (29-81)**
Aetiology of AP			
Alcohol	126 (73)	25 (65)	25 (86)
Biliary	28 (16)	10 (26)	2 (7)
Idiopathic or other	18 (10)	3 (8)	2 (7)
Onset of symptoms (h)*	24 (2-72)	24 (4-72)	24 (2-72)
CRP (mg/L)*	22 (3-369)	20 (3-383)	27 (3-414)
Creatinine (µmol/L)*	65 (40-207)	71 (46-313)	85 (48-1,066)
SOFA*	0 (0-9)	0 (0-8)	4 (0-15)
APACHE II*	5 (0-13)	6 (0-14)	7 (0-21)
MMS *	0 (0-3)	0 (0-3)	0 (0-5)
MMS < 2	172(100)	33 (87)	21 (72)
MMS ≥ 2 o	0 (0)	5 (13)	8 (28)
Renal replacement therapy	0 (0)	0 (0)	15 (52)
Mechanical invasive ventilation	1 (0.6)	4 (11)	25 (86)
Hospital days*	4 (0-98)	11 (3-20)	27 (1-108)
Mortality	1 (0.6)	1 (3)	6 (22)

Data are presented as *median (range) or number and (%). APACHE II was determined within 24 h of admission using the most abnormal value for each physiological variable. APACHE acute physiology and chronic health evaluation, CRP C-reactive protein, MMS modified Marshall score, SOFA sepsis-related organ-failure assessment score.

3.2.2 CLASSIFICATIONS AND DEFINITIONS

BDI classification and grade

BDIs type were classified according to the Amsterdam criteria (Bergman et al., 1996). Severity of bile leak was graded from endoscopic images as LG if the leak was visible after intrahepatic opacification from the distal part of the common bile duct and HG if the leak became visible in cholangiography before intrahepatic opacification (Dolay et al., 2010).

BDI healing

Healing of the bile duct leak was diagnosed when a patient was symptomless without visible bile in the drains, and no adverse events appeared. Bile duct strictures were diagnosed as healed when there was no visible stricture present in the control ERC when the stents were removed.

PEP

The criteria used for PEP diagnostics were a new onset of typical epigastric abdominal pain after ERCP lasting at least 24 h, with elevated plasma pancreas-specific amylase or amylase levels more than 3 times the upper reference limit and/or typical diagnostic findings in CT or MRI (Cotton et al., 2009). PEP was further graded as mild, moderately severe, or severe AP according to the Revised Atlanta Classification (Sarr et al., 2013), and as mild, moderate, or severe according to Cotton's Classification (Cotton et al., 2009).

AP

AP diagnostic criteria were persistent typical epigastric abdominal pain with elevated plasma pancreas-specific amylase or amylase at least three times the upper reference limit and/or typical diagnostic findings of AP in CT or MRI. AP was graded according to the Revised Atlanta Classification as mild (without OD or systemic complications), moderately severe (with transient OD that resolves within 48 h, and/or local/systemic complications), or severe (with persistent OD lasting more than 48 h) (Sarr et al., 2013). At the time of admission to hospital, the presence of OD was assessed according to Modified Marshall Score. The score of three organ groups (respiratory, renal, and cardiovascular) is evaluated, and each of these organ groups can receive a score of 0 - 2, depending on the level of organ function. A total calculated sum of scores ≥ 2 indicates OD (Marshall et al., 1995). OD is determined as transient if it resolves within 48 h, and as persistent if it persists over 48 h.

3.2.3 SAMPLING AND ANALYTICAL METHODS

In study II, plasma pancreas-specific amylase or amylase levels were analysed before and 4 h after ERCP to identify the possible development of PEP. If patients remained in hospital overnight, plasma pancreas-specific amylase was also measured 24 h after ERCP.

In study III, we analysed plasma or pancreas-specific amylase, bilirubin, urine T-2 and urine T-2 with a dipstick in all patients before ERCP and 4 h after ERCP. If a patient stayed overnight, 24 h tests were also taken. Pancreas-specific amylase and plasma amylase were measured according to hospital routine with an Abbott Architect analyser. Urine T-2 was measured by a quantitative time-resolved immunofluorometric assay (IFMA). A urine T-2 dipstick test (Actim Pancreatitis; Medix Biochemica, Kauniainen, Finland) was dipped into the fresh urine sample, and the result was detectable within 5 minutes. The dipstick test has a detection line and reference line, which both turn to blue if the urine T-2 concentration exceeds 50 μg/l, indicating a positive dipstick test.

In study IV, we measured serum SPINK1, T 1-3, trypsin-2-AAT, plasma pancreas-specific amylase or amylase, creatinine, and CRP 0-12 h after admission to hospital in all AP patients. Serum was separated and stored until analysed at -20 °C. SPINK1**,** T 1-3, and trypsin-2-AAT were quantified by monoclonal antibodies-based time-resolved immunofluorometric assays. Plasma pancreas-specific amylase or amylase, creatinine and CRP concentrations were analysed as hospital routine blood samples with a Modular P clinical chemistry analyser (Hitachi, Tokyo, Japan) using a Pancreatic-Amylase liquid kit, an Alpha-Amylase liquid kit, a CREA plus kit and a Tina-quant CRPL3 kit respectively.

3.2.4 STATISTICAL ANALYSIS

Statistical analyses were performed on SPSS version 22.0 (SPSS, IBM Corporation, Somers, NY, USA) statistical software and in study III also Analyse-it software for Microsoft Excel 2016 (version 4) was used. The data is presented as median and range, or number and percentage. Comparisons were made between groups by Chi-square or Fisher's exact tests for categorical variables, and continuous or ordinal variables were compared by the Wilcoxon-Mann-Whitney *U* test or the Kruskal-Wallis test. Comparisons between three ordered groups were made using the Jonckheere-Terpstra trend test. Subgroup-analyses were performed using logistic regression analysis. To identify potential confounding variables, all the subgroups with $P < 0.05$ in the univariate analysis were selected for a multivariate logistic regression analysis.

The agreement between the quantitative urine T-2 test and the dipstick test was evaluated in study III with kappa statistics ($\kappa < 0.20$ indicates poor agreement, and $\kappa > 0.81$ very good agreement) (Landis et al., 1977).

To clarify the predictive value of a biomarker in study IV, a receiver operator characteristic (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated. AUC 1.0 is the best result for a test, indicating 100% specificity and sensitivity, and 0.5 indicates no discriminatory power. Optimal cut-off value was determined from the ROC curve to obtain 93% specificity and the corresponding sensitivity for each biomarker. The positive and negative likelihood ratio and diagnostic odds ratio with 95% confidence intervals were calculated for these cut-off values (Glas et al., 2003, Newcombe, 1998).

3.3 RESULTS

3.3.1 STUDY I: ERCP IN MANAGEMENT OF BDI

The majority of the total of 99 patients had Amsterdam type A BDIs (74 patients, 75%), and the leak was most frequently found in the cystic duct (60 patients, 64%). The type and treatment of the BDI are presented in Table 11.

Table 11 *Characteristics of BDI and treatment*

	Number of patients (%)
Type of BDI (Amsterdam classification)	
A	74 (75)
B	17 (17)
C	4 (4)
D	4 (4)
Bile duct leak	94 (95)
Location of leak	
Cystic duct	60 (64)
Duct of Luschka or peripheral radicals	21 (22)
Common bile duct	13 (14)
Grade of the leak	
HG	43 (46)
LG	51 (54)
Common bile duct stones	17 (17)
Bile duct stricture	12 (12)
Location of stricture	
Common bile duct	8 (67)
Common hepatic duct	2 (17)
Right hepatic duct	2 (17)
Endoscopic management	
Sphincterotomy	56 (57)
Sphincterotomy and biliary stent	37 (37)
Biliary stent without sphincterotomy	1 (1)

BDI= bile duct injury; HG = high-grade; LG = low-grade

Management of Amsterdam type A BDIs

All 74 patients with Amsterdam type A BDI were treated successfully in ERC. Of these patients, we further explored a group of 71 patients with native papillae who were treated with ES alone (ES group, 50 patients) or with ES with stenting (EST group, 21 patients). We compared the success of primary treatment between these groups and found no difference in the outcomes (P= 0.951). The results did not differ in the closure time of the leak (P = 0.179), discharge time from the hospital (p = 0.298) or in primary healing rate in LG leaks (P = 1,000) and HG leaks (p = 1,000) (Table 12). However, more ERCs per patient were needed in the EST group than in the ES group as the stents needed to be removed in an additional ERC (2.1 [mean] vs 1.2, respectively, P = 1.000). Five patients (10%) in the ES group needed additional ERCs and stenting before healing. Two patients (10%) in EST group needed secondary stenting before the leak closed. We found no difference in the efficiency of these two treatment modalities.

Management of Amsterdam type B, C, and, D BDIs

Seventeen patients presented Amsterdam type B leak in ERC. The leak originated from the common bile duct in four patients, the main hepatic duct in five patients, and the right hepatic duct in one patient. Stricture with a concomitant cystic stump leak was present in 7 patients. Endoscopic treatment with stenting was successful in 14 out of 17 patients in this group. Three patients needed operative treatment.

All four patients with Amsterdam type C injuries (strictures) were successfully treated with multiple plastic or covered self-expandable metallic stents in ERC. No operative treatment was needed for this group.

All ERCs in patients diagnosed with an Amsterdam type D injury remained diagnostic. They all underwent surgery and a Roux-en-Y hepaticojejunostomy.

Outcome of the treatment

Endoscopic treatment succeeded in 93% of all BDI patients. Two patients (2%) had PEP (mild and moderately severe according to the Atlanta classification). There were no cases of bleeding, perforation, cholangitis, or mortality related to ERC. Patients undergoing surgery had no long-term complications.

Table 12 *Comparison of therapy of Amsterdam A BDI treatment between the endoscopic sphincterotomy (ES group) and endoscopic sphincterotomy and stenting group (EST group)*

	ES group n = 50	EST group n = 21	*P*
Patient and injury characteristics			
Female	27 (54)	12 (57)	0.808 a
Age (years)*	57 (27-88)	66 (32-93)	0.053 b
Type of leak			
Cystic stump	32 (64)	16 (76)	0.316 a
Duct of Luschka	18 (36)	5 (24)	0.316 a
LG/ HG leak	35/15 (69/31)	12/9 (57/43)	0.296 a
Endoscopic treatment and outcome			
1.ERC success	45 (90)	19 (90)	0.951 a
2.ERC	5 plastic stents	19 stent removal 1 stone removal and stenting 1 stent change	
3.ERC	5 stent removal 1 nasobiliary stent	1 stent removal 1 diagnostic 1 additional stent	
4. ERC		1 stent removal	
Total number of ERCs	60	46	0.000 c
Drainage days after ERC *	4 (0-21)	3 (1-14)	0.179 c
Hospitalisation days after ERC *	4 (1-53)	4 (1-65)	0.298 c
Stent days *	40 (31-63)	54(29-297)	0.338 c
Follow-up time, months*	51 (1-129)	28(1-116)	0.028 c
Post ERCP pancreatitis	2 (4)	0 (0)	0.353 a

Data are presented as *median (range) or numbers and (%). BDI biliary duct injury; a Chi-square test; b Unpaired independent t-test; c Wilcoxon- Mann- Whitney test, LG low-grade leak, HG high-grade leak

3.3.2 STUDY II: DICLOPHENAC IN PREVENTION OF PEP

In this study, 97% of the 2,000 ERCP cases were therapeutic, and the most frequent procedures were biliary ES (in 1,327 patients [66%]), biliary stent placement (in 749 patients [37%]) and biliary stone extraction (in 459 patients [23%]). Slightly more than half the patients had native papillae (1,058 patients, [53%]) and cannulation was ranked as difficult on 328 occasions (31%). The median cannulation time in native papillae cases was 92 s, and the median total time of the procedure was 21 minutes in all patients as well as in native papilla cases. Two study groups, DG and CG, did not differ in their patient characteristics or the incidence of PEP, which was 2.8% in both the groups and also in the entire cohort. Furthermore, there was no difference between the groups in the severity of PEP.

We found no subgroup that would have benefitted from rectal diclofenac (Table 13). In univariate analysis, the risk factors for PEP were native papilla, pancreatic ES, pancreatic brush cytology, papillectomy, pre-cut ES, difficult cannulation, and a prolonged procedure time. A pair-wise logistic regression of these variable with diclofenac, showed no effect of diclofenac in the development of PEP. In a multivariate analysis, significant factors associated with the risk of PEP were pancreatic ES, pancreatic brush cytology, difficult cannulation, and a prolonged procedure time. Diclofenac showed no effect on the risk of PEP (Table 14).

Table 13 *Comparison of incidence of post ERCP pancreatitis (PEP) risk factors between the study groups*

Risk factors	Control group n/N (%)	Diclofenac group n/N (%)	*P*
Female gender	13/408 (3.2)	14/416 (3.4)	0.889
Native papillae	21/535 (3.9)	19/523 (3.6)	0.803
Female	12/241 (4.9)	13/249 (5.2)	0.900
Female < 40 years	1/24 (4.2)	0/28 (0)	0.462*
Difficult biliary cannulation	12/156 (7.7)	10/150 (6.7)	0.747
Previous pancreatitis	2/137 (1.5)	2/142 (1.4)	1.000*
Previous PEP	1/16 (6.3)	0/6 (0)	1.000*
Duration of ERCP > 40 min	12/145 (8.2)	9/169 (5.3)	0.330
Normal serum bilirubin	14/457 (3.1)	10/485 (2.1)	0.330
ERCP procedures			
Pancreatic sphincterotomy	17/192 (8.9)	12/158 (7.6)	0.671
Biliary sphincterotomy	21/500 (4.2)	13/468 (2.8)	0230
Pancreatic duct dilatation	5/108 (4.6)	4/120 (3.3)	0.739*
Pancreatic stent placement in chronic pancreatitis	6/239 (2.5)	10/258 (3.9)	0.389
Pancreatic brush cytology	8/158 (5.2)	6/194 (3.1)	0.335
Pancreatic duct opacification	10/322 (3.1)	14/333 (4.2)	0.471
Pre-cut	1/15 (6.7)	2/18 (11.1)	1.000*
Papillectomy	1/12 (8.3)	3/12 (25)	0.590*

*Data are presented as numbers and (%) n number of pancreatitis cases. N group size; *Fisher's exact test; ERCP endoscopic retrograde cholangiopancreatography*

Table 14 *Logistic regression analysis in the effect of diclofenac on the risk of PEP, when adjusting for the risk of PEP in a univariate analysis*

	OR	(95%CI)	*P*
Diclofenac administration	1.031	(0.60- 1.79)	0.913
Pre-cut	1.125	(0.29- 4.39)	0.865
Native papilla	1.099	(0.53- 2.28)	0.800
Papillectomy	2.818	(0.788- 10.08)	0.111
Difficult biliary cannulation	2.145	(1.10- 4.20)	0.026
Pancreatic cytology	3.189	(1.233- 8.250)	0.017
Pancreatic sphincterotomy	2.857	(1.49- 5.47)	0.002
Duration of procedure	1.020	(1.01- 1.03)	0.001

OR odds ratio; CI confidential interval

3.3.3 STUDY III: URINE T-2 DIPSTICK TEST IN PEP DIAGNOSIS

Of the 400 patients in this cohort, PEP developed in 15 cases (3.8%). There were no severe PEPs among these patients; 14 had mild PEP, and one was diagnosed as moderately severe according to the Atlanta classification.

A baseline dipstick test was taken for all 400 patients, and it was negative in 279 patients. The 4 h test for these was positive in 58 patients, including 6 PEP cases. In 214 cases with the negative 4 h dipstick test, 6 developed PEP, and 3 of these patients had a positive 24 h test, while 3 tests were missing. In all cases, the negative dipstick test excluded PEP (Table 15).

In all patients, regardless of the baseline results, the 4 h dipstick test was negative for 240 of 388 patients, and the 24 h test in 154 of 269 patients. The 4 h dipstick test was positive in 139 (35%) patients without PEP. However, none of the patients with both negative 4 and 24 h dipstick tests developed PEP.

When the dipstick test was evaluated with persistent abdominal pain symptoms (diagnostic criteria for PEP), the accuracy of diagnostics improved: 4 h after the ERCP dipstick test sensitivity was 60%, specificity 99%, the positive predictive value 71%, and the negative predictive value 98%. The 24 h dipstick test sensitivity was 100%, specificity 98%, the positive predictive value 71% and the negative predictive value 100% (Table 16).

We also measured urine T-2 quantitative concentrations and compared them with dipstick performance. The kappa value 4 h after ERCP was 0.828, and 24 h after ERCP it was 0.773. The concentrations of urine T-2 increased significantly during this time.

Table 15 *Urine trypsinogen-2 dipstick results*

	Positive (%)	False positive (%)	Negative (%)	False negative (%)	Missing (%)
All patients					
Before ERCP	121 (30)	121 (30)	279 (70)	0	0
4 h after ERCP	148 (37)	139 (35)	240 (60)	6 (1.5)	12 (3)
24 h after ERCP	115 (29)	109 (27)	154 (39)	0	131 (33)
PEP patients					
Before ERCP	2 (13)	2 (13)	13 (87)	0	0
4 h after ERCP	9 (60)	0	6 (40)	6 (40)	0
24 h after ERCP	9 (100)	0	0	0 (0)	6 (40)
Patients with abdominal pain after ERCP					
Before ERCP	4 (17)	4 (17)	20 (83)	0	0
4 h after ERCP	14 (58)	5 (21)	10 (41)	6 (25)	0
24 h after ERCP	14 (58)	5 (21)	4 (17)	0	6 (25)

ERCP endoscopic retrograde cholangiopancreatography; False positive test = positive test without diagnosed PEP; False negative test = negative test in patient with diagnosed PEP

Table 16 *Diagnostic performance of urine trypsinogen-2 dipstick test with abdominal pain detecting PEP*

	Sensitivity (95% CI) %	Specificity (95% CI) %	PPV %	NPV %
4 h dipstick positive	60 (35-80)	99 (97-99)	64	98
24 h dipstick positive	100 (70-100)	98 (96-99)	71	100

PPV positive predictive value; NPV negative predictive value; CI confidential interval

3.3.4 STUDY IV: SPINK1, T 1 - 3, AND TRYPSIN-2-AAT IN PREDICTING SEVERE AP

According to the Atlanta criteria, AP was diagnosed as mild in 172 (72 %), moderately severe in 38 (16 %), and severe in 29 (12 %) cases. The great majority of patients (n = 226, 94%) presented without OD on admission to hospital. Thirteen patients (5.4 %) had OD upon arrival, and they were diagnosed further as transient OD in 5 patients, and persistent OD in 8 patients. The duration of symptoms did not differ between patients with or without OD on admission (P = 0.05). OD developed after admission in 27 patients; in 11 patients, OD was transient, as it was resolved within 48 h, but in 21 patients, OD was persistent, as it lasted over 48 h.

Eight patients (3.3%) died during hospitalization, and 6 of these had severe AP. Five of the deaths occurred during the first week in hospital, and the three others on hospital days 13, 24 and 25. Two patients with SAP died within 24 h of admission, and they were not treated in ICU due to their high age and severe comorbidities. One patient with mild AP also died 24 h after admission: he committed suicide on the ward. One patient with moderately severe AP died on day 13 on the ward, and because the relatives prohibited an autopsy, the cause of death remained unclear. The other 4 dead patients had SAP.

Predictors of severe AP on admission

Serum levels of SPINK1, T-1 and 2, trypsin-2-AAT and creatinine correlated on admission with the severity of AP (P < 0.05); T3, CRP and amylase levels did not (Table 17). In detecting severe AP, SPINK1 was most accurate, having the largest AUC value (0.779 [SD, 0.054]), followed by T-2 (0.772 [SD, 0.049]), creatinine (0.719 [SD 0.054]), trypsin-2-AAT (0.690 [SD, 0.045]), T-1 (0.656 [SD, 0.054]), T-3 (0.600 [SD, 0.059]) and CRP (0.565 [SD, 0.062]).

With an optimal cut-off value of 196 µg/l giving high 93% specificity, SPINK1 had a sensitivity of 48% and a DOR 11.3, and at a cut-off of 1,512 µg/l T-2 had a 93% specificity, a sensitivity of 38% and a DOR of 6.5 in diagnosing severe AP in all patients on admission (Table 18). Male gender, creatinine, SPINK1, T-1, T-2 and T-3 were associated with SAP in univariate logistic regression analysis. However, only SPINK1 was an independent predictor for OD.

Table 17 *Concentrations of biomarkers in patients with mild, moderately severe and severe acute pancreatitis*

Reference range	**Mild AP (n = 172)**	**Moderately severe AP (n = 38)**	**Severe AP (n = 29)**	***P*[a]**
SPINK1 3 - 16 µg/l*	41 (28-70)	51 (35-179)	176 (59-325)	0.000
Trypsinogen-1 7.2 - 49 µg/l*	316 (127-551)	404 (210-953)	451 (300-451)	0.001
Trypsinogen-2 2.6 - 18 µg/l*	254 (107-618)	582 (295-1100)	1186 (440-1844)	0.000
Trypsinogen-3 <4.4 µg/l*	13 (5-25)	18 (3.3-38.5)	20.5 (8-52)	0.081
Trypsin-2-AAT 2.3 - 12 µg/l*	93 (16-232)	198 (76-425)	258 (132-45)	0.000
Creatinine 50 - 100 µmol/l*	65 (56-78)	72 (57-72)	84.5 (65-162)	0.000
CRP < 10 mg/l*	22 (5-92)	24 (6-24)	27 (10-27)	0.263
Pancreatic amylase 10 - 65 U/l*	360 (181-1026)	657 (315-1629)	463 (203-785)	0.070

Data presented as median (interquartile range)
AP acute pancreatitis; CRP C-reactive protein; T-2-AAT complex between trypsin-2 and α_1-antitrypsin
*Reference values for healthy individuals for trypsinogen-1, trypsinogen-2, trypsinogen-3, SPINK1, and complex between trypsin-2 and α_1-antitrypsin (Itkonen et al., 2012, Hedstrom et al., 1994, Oiva et al., 2011).
[a]Jonckheere-Terpstra for trend

Predictors of severe AP in patients without OD on admission

SPINK1 was the most accurate predictor for development of severe AP after admission, with the largest AUC value (0.742 [SD, 0.062]) followed by T-2 (0.726 [SD, 0.063]), trypsin-2-AAT (0.657 [SD, 0.056]), creatinine (0.656 [SD, 0.063]), T-1 (0.652 [SD, 0.071]), T-3 (0.557 [SD, 0.069]) and CRP (0.499 [SD, 0.075]). In finding patients developing severe AP at high 93% specificity, SPINK1 and T-2 had the best diagnostic accuracies; with a cut-off of 166 μg/l SPINK1 had a sensitivity of 48% and a DOR of 11.52; with a cut-off 1375 μg/l, T-2 had a sensitivity of 38% and a DOR of 7.8. (Table 18)

SPINK1, T-1, and T-2 were significant predictive markers of SAP in univariate logistic regression analysis, but in multivariate logistic regression analysis SPINK1 was the only independent predictor of SAP.

Table 18 *Performance of biomarkers in diagnosis of severe acute pancreatitis*

	Cut-off	AUC (95% CI)	Sensitivity (95% CI), %	Specificity (95% CI), %	+LR (95%CI)	-LR (95%CI)	DOR (95% CI)
All patients (n = 239)							
Trypsinogen-1, µg/l	1,279	0.656 (0.550-0.762)	19 (9-38)	93 (87-94)	2.6 (1.1-6.0)	0.9 (0.71-1.0)	3.0 (1.06-8.26)
Trypsinogen-2, µg/l	1,512	0.772 (0.676-0.868)	38 (20-50)	93 (89-96)	4.7 (2.4-9.3)	0.7 (0.6-0.9)	6.5 (2.7-15.9)
Trypsinogen-3, µg/l	56	0.600 (0.485-0.715)	24 (12-42)	93 (89-96)	3.4 (1.5-7.6)	0.8 (0.7-1.0)	4.1 (1.5-11.2)
SPINK1, µg/l	196	0.779 (0.683-0.876)	48 (31-66)	93 (87-95)	6.3 (3.5-11.6)	0.6 (0.4-0.8)	11.3 (4.7-27.5)
Trypsin-2-AAT, µg/l	785	0.690 (0.601-0.778)	17 (8-35)	93 (89-96)	2.4 (0.95-6.15)	0.89 (0.75-1.06)	2.7 (0.9-8.1)
Creatinine, µmol/l	111	0.719 (0.614-0.825)	33 (20-52)	93 (88-95)	4.53 (2.27-9.00)	0.70 (0.54-0.93)	6.4 (2.5-16.0)
Patients presenting without OD on admission (n = 226)							
Trypsinogen-1, µg/l	1,278	0.652 (0.513-0.790)	22 (11-45)	93 (88-95)	3.05 ‘(1.24-7.49)	0.83 (0.65-1.05)	3.69 (1.20-11.39)
Trypsinogen-2, µg/l	1,375	0.726 (0.602-0.850)	38 (21-59)	93 (88-96)	5.20 (2.51-10.82)	0.67 (0.48-0.94)	7.80 (2.80-21.74)
Trypsinogen-3, µg/l	55	0.557 (0.422-0.691)	21 (11-43)	93 (88-95)	3.25 (1.13-8.06)	0.82 (0.65-1.05)	3.96 (1.27-12.30)
SPINK1, µg/l	166	0.742 (0.620-0.864)	48 (28-68)	93 (88-96)	6.50 (3.36-12.62)	0.57 (0.38-0.85)	11.52 (4.22-31.45)
Trypsin-2-AAT, µg/l	785	0.657 (0.548-0.767)	19 (8-40)	93 (88-96)	2.60 (0.95-7.13)	0.87 (0.71-1.08)	2.99 (0.89-9.99)
Creatinine, µmol/l	103	0.656 (0.531-0.780)	19 (8-40)	93 (89-96)	3.00 (1.08-8.39)	0.86 (0.7-1.07)	3.48 (1.02-11.84)

AUC area under the curve; DOR diagnostic odds ratio; LR likelihood ratio; OD organ dysfunction; Trypsin-2-AAT complex between trypsin-2 and α_1-antitrypsin.

3.4 DISCUSSION

3.4.1 ERCP IN TREATMENT OF BDI

ERCP has become the main treatment of choice in BDIs in recent decades as equipment, and especially stents, have improved. However, treatment of BDI is a rather rare event in endoscopic units, and there is lack of large prospective studies of this subject. The majority of studies have explored the most usual type of BDI, the Amsterdam type A leak. The guidelines for treatment of BDI strictures originates mostly from series exploring other benign types of biliary stricture as well as the recommendations on the treatment of major leaks of the common bile duct are mainly based on studies of leaks after liver surgery. In this cohort of 99 patient, we had mostly Amsterdam class A injuries, and the number of other injury types remained too small for more than an observational report of cases.

The ESGE guideline recommends endoscopic placement of plastic stents in the management of bile duct leaks minor to total transection of common bile duct or common hepatic duct (J. M. Dumonceau et al., 2018). This recommendation is based on three RCTs of 27, 52, and 63 patients, which showed that endoscopic stenting is superior to single ES, that endoscopic stenting with or without ES are equally efficient treatments and that the size of the stents (7 or 10 Fr plastic stents) has no effect on the final outcome (Dolay et al., 2010, Mavrogiannis et al., 2006, Katsinelos et al., 2008). In the event of a refractory bile leak, ESGE recommends SEMS, because a comparative study of 40 patients has shown FC-SEMSs to be superior to multiple plastic stents (Canena et al., 2015).

Our present study's most important finding was that ES alone and ES with stenting had equal 90% success in biliary leak closure, which differs from the ESGE guideline's recommendations and most previous studies. The RCT, which is the cornerstone of the ESGE recommendation, showing the superiority of biliary stenting in the treatment of biliary leaks, included only 27 patients. However, our observational retrospective study consisted of 71 patients with dominant ES group of 50 patients. In addition to our study, two other studies of 31 and 207 patients demonstrate the effectiveness of biliary ES in the treatment of Amsterdam type A LG leaks (Aksoz et al., 2009, Sandha et al., 2004). We found no difference in the treatment of Amsterdam type A low-grade or high-grade patients. The reason to our success may be explained by the ES technique: in our clinic, we perform ES by cutting the sphincter completely, extending the incision to the maximum safe limit. Proper ES balances the pressure gradient between the bile duct and bowel, and enables free bile flow to the bowel, ending the leak. A smaller ES does not sufficiently balance the pressure gradient, a technical detail which may explain the difference between our and previous published results. There was need for additional stenting in 10% of both study groups. In the ES group, the failure

may be explained by insufficient ES: in the EST group, the plastic stent position may have been suboptimal, failing to balance the pressure gradient. Although the ES and EST groups have similar success rates, ES is a cost-effective procedure because it does not require an additional endoscopy and stent removals: in the ES group, the mean number of procedures was 1.2; in the EST group, it was 2.2.

3.4.2 PREVENTION OF PEP

The European and Japanese societies of Gastroenterology recommend routine use of diclofenac for all ERCP patients without contraindications (J. Dumonceau et al., 2014, Mine et al., 2017). These guidelines are based on several meta-analyses between 2009 and 2014 dealing with RCTs showing a mostly positive effect on diclofenac and indomethacin in reducing PEP. The number of patients in these RCTs varies from 100 to 602 (Khoshbaten et al., 2008, Elmunzer et al., 2012), and the number of patients in meta-analyses varies between 912 and 2,269 (Elmunzer et al., 2008, Ding et al., 2012). The majority of these studies have included only patients with high PEP risk, and thereafter the incidence of PEP in these studies has reached 26% (Khoshbaten et al., 2008). However, three recent RCTs (two studies of indomethacin and one study of diclofenac) reported controversial results, showing no role of rectal NSAIDs in preventing PEP in consecutive ERCP patients. They did show, however, the effect of NSAIDs in high-risk patients. These studies included 144, 449, and 665 patients, and the incidences of PEP in these studies were 7.6%, 6.0 %, and 6.3% respectively (Lua, Muthukaruppan & Menon, 2015, Levenick et al., 2016, Dobronte et al., 2014). Based on these findings, the American Endoscopic Society recommends NSAIDs only for average and high PEP risk patients, not for consecutive patients as European and Japanese guidelines do (ASGE Standards of Practice Committee et al., 2017, J. Dumonceau et al., 2014, Mine et al., 2017).

Indomethacin has been shown to be a more effective PLA2 inhibitor than diclofenac, and theoretically it may be superior in PEP prevention (Makela et al., 1997). However, there is lack of studies comparing the effects of diclofenac and indomethacin in preventing PEP (Lyu et al., 2018).

Our results with a low PEP risk of 2.8% in both the study arms differ from majority of previous published results. We researched consecutive patients in a high-volume centre, including the feasible number of high-risk patients as well. We found no preventive or attenuating effect of diclofenac on PEP in low-risk patients or in different high-risk subgroups. Similarly, three RCTs (Lua et al. 2015, Levenick et al. 2016, Dobronte et al. 2014) found no effect on diclofenac in preventing PEP in low-risk patients. However, our study differs from these in their retrospective nature, the lower rate of PEP and a larger number of patients. These results indicate that the role of NSAIDs in

preventing PEP remains unclear, and further large scale RTCs are needed to explore the use of NSAIDs for all patients.

3.4.3 DIAGNOSIS OF AP

Large proportions of trypsinogens are released to the systemic circulation and urine in the early phase of AP. Serum and urine concentrations increase within a few hours after the onset of the disease. This enables the use of trypsinogens, SPINK1 and trypsin-2-AAT in diagnosis and in the severity assessment of AP (Lippi, Valentino & Cervellin, 2012b).

Urine T-2 dipstick in diagnosis of PEP

Urine T-2 has been shown to be as effective in the diagnosis of AP as a serum amylase and lipase test (Rompianesi et al., 2017). Only four studies have evaluated the use of T-2 in the diagnosis of PEP, and three of these involved a dipstick test. The size and number of PEP cases in these previous studies was smaller than ours; there were 11 PEP cases of a total of 106 patients in the study of Kemppainen et al. (E. Kemppainen et al., 1997), 4 PEP cases of 29 patients in the study of Sankaralingam et al. (Sankaralingam et al., 2007), and 13 PEP cases of 150 patients in the study of Tseng et al. (Tseng et al., 2011). In the studies of Sankaralingam and Tseng, all patients with a positive base-line dipstick test were excluded. Kemppainen et al. did not test the pre-ERCP dipstick test; instead, they measured urine T-2 concentrations before ERCP and excluded all patients with high trypsinogen levels. In our study, we had a surprisingly high proportion (33%) of positive pre-ERCP dipstick tests. Many conditions and common indications for ERCP, such as biliary tract malignancies, biliary stones, and biliary and hepatic inflammations, increase T-2 in urine (Itkonen, 2010, Hedstrom et al., 1996). However, we reasoned that in clinical practice, pre-ERCP dipstick tests are not taken, and we therefore included all patients in our analysis, regardless of the baseline dipstick result.

In our entire cohort, we found the dipstick test less accurate than previous studies in the diagnosis of PEP. However, our result was similar to others when we also only analysed patients with a negative pre-ERCP dipstick test. Since abdominal pain is an important diagnostic criterion of PEP and needs to be evaluated in PEP diagnostics when amylase is used as a diagnostic test, we also evaluated dipstick test results with abdominal symptoms. This increased the accuracy of the dipstick test by up to 100% for sensitivity and 98% for specificity. Although the urine dipstick test indicates positive dipstick results in many non-PEP cases, in this study, we found that a negative test rules out, and a positive test with abdominal pain symptoms detects, PEP with a high degree of accuracy.

Serum levels of SPINK1, T 1 - 3, and trypsin-2-AAT in prediction of SAP

Our major finding in this study was that SPINK1 identified nearly half the patients who presented without OD on admission but developed SAP later. Recognition of these patients is important because in SAP, two out of three patients develop OD early during the course of the illness, and up to 50% of deaths occur within the first two weeks (Padhan et al., 2018). Since a specific pharmacological treatment is lacking and the best results are achieved in early intensive care, these patients need to be recognised in the disease's early stage. This diagnostic challenge has been studied since the Ranson score was developed in 1974, but all scores and biomarkers to date have limitations in their use. In recent years, there have been attempts to find a single ideal biochemical parameter which could precisely predict the severity of AP in the early course of the disease.

SPINK1, T 1-3 and trypsin-2-AAT have previously been studied in the diagnosis of AP and as predictors of OD (Sainio et al., 1996b, Hedstrom et al., 1996, Lempinen et al., 2003, Ogawa, 1988b, Oiva et al., 2011). We made similar observations to these earlier studies: concentrations of SPINK1, T 1-3, and trypsin-2-AAT increases in AP patients; SPINK1, T-1 and 2 and trypsin-2-AAT concentrations increase with AP severity. They are, thus useful in the diagnosis of AP. However, our study differs from these earlier studies because we used the revised Atlanta classification, our cohort size was bigger and we analysed patients who presented without OD. A similar protocol has been used in studies evaluating the utility of assays of cytokines, nucleosomes and soluble CD73 for the development of severe AP in patients presenting without OD (Nieminen et al., 2014, Maksimow et al., 2014, Penttila et al., 2016). These markers provide sensitivities varying between 26–36% in high specificities of 93-96%, which is very comparable with our results for T-2 (38% sensitivity and 93% specificity) and SPINK1 (48% sensitivity and 93 % specificity).

Of the markers studied here, T-2 and SPINK1 are of especially great interest for their potential use in the diagnosis of AP and predicting the development of OD. SPINK1 is already available in our laboratory repertoire as a tumour marker (TATI). It may therefore at least have the potential to be used in emergency rooms in the differentiation of patients prone to developing SAP.

3.4.4 FUTURE ASPECTS

We have questioned the guidelines for treatment of BDI Amsterdam type A leaks and the prevention of PEP with rectal diclofenac in our retrospective and relatively sizable studies.

The Amsterdam type A leak, as one of the most frequently seen complications of cholecystectomy, requires a straightforward and cost-effective method of treatment. Our result for Amsterdam type A leak treatment shows that ES without stenting is as effective as ES with stenting, and the number of endoscopies needed for the treatment with ES alone is half those for stenting. A lack of larger-scale RCTs makes our study more significant, and we can thus recommend a consideration of ES as a first treatment method in Amsterdam type A BDIs. Biodegradable stents have recently been studied in Amsterdam type A BDI treatment, and they have shown equally good results to plastic stents (Siiki et al., 2018). These stents seem effective, and no additional endoscopy is needed because they degrade. However, the price of the stent makes this treatment method more expensive than a single ES. Further larger-scale RCTs are therefore needed to compare the effectiveness and costs of ES, with and without biodegradable stents. The scarce cases of BDIs in endoscopic units and the emergency setting around these patients complicates the conduct of well-organised large-scale RTCs. In the HUH Endoscopic Unit, of 1,500 annually performed ERCPs, only an average of 10 BDI cases appears every year. This may explain the limited series of former RCTs. Multi-centre studies may be an answer to this problem.

PEP in its worse outcome is a devastating complication after ERCP. The discovery of a perfect preventive medication for PEP should still be the focus of future studies. It seems that NSAIDs may not have the preventive power we had hoped, since our study, with 3 RCTs, questions the use of diclofenac in the prevention of PEP in low-risk patients. Further randomised trials in a low-risk unit, as well as in low risk procedures, are needed to explore this subject.

None of the agents studied in PEP prevention have been as promising, harmless and easy to use as NSAIDs. We still need to identify new agents and technical aspects that could be studied further in PEP prevention. Our study shows low PEP incidence and therefore indicates that many technical details may be conducted differently to prevent PEP. We use methods recommended in ESGE and ASGE guidelines such as guidewire-guided cannulation, the avoidance of the pancreatic duct opacification and early biliary pre-cut to prevent PEP (J. Dumonceau et al., 2014, ASGE Standards of Practice Committee et al., 2017). Furthermore, we use propofol-anaesthesia during all ERCPs, which calms the operating environment; when the patient is still and relaxed, all the procedure's risks are smaller. ERCPs should also be concentrated in centres with feasible volumes: the more experienced endoscopists are, the smaller the PEP rate (Keswani et al., 2017). All these

technical aspects need further exploration, and they should be considered for adoption in clinical use in endoscopic units.

Although diagnosis and treatment of AP has been intensively studied worldwide, new and useable tools for diagnosis have not been found and the overall mortality of the disease has not improved significantly (Agarwal et al., 2016). In assessing the severity of AP, CRP has remained the most frequently used marker, although it is far from being an optimal indicator. In our study, after amylase, CRP was the worst predictor of SAP, strengthening the hope of the development of a better clinical marker. SPINK1 is accurate in the diagnosis of AP, but since it detects only half the cases where OD develops, further improvement in its sensitivity in predicting the development of SAP is desirable. OD can develop after several days, and it therefore would be important to explore whether daily determinations of SPINK1 would improve the detection of OD. It is also known that serum concentrations of trypsin-2-AAT continue to increase after the onset of the disease for at least 5 days, and it would also be interesting to explore its performance 48 and 72 h after admission. T-2 had the second-best result in our cohort in predicting OD. It is an interesting enzyme, because its concentration remains elevated for at least 5 days and is higher in urine than in serum (E. A. Kemppainen et al., 1997). It would be interesting to use a similar study protocol to research how urine T-2 would predict SAP. A recent Japanese study of urine T-2 concentrations showed that it might be a suitable marker to determine extra-pancreatic inflammation in AP (Yasuda et al., 2019).

In the diagnosis of PEP, the urine T-2 dipstick test performed well diagnostically. It is cheap and easy to use, and could be used more widely clinically worldwide in endoscopic units in the diagnosis of PEP. However, more large-scale prospective studies are needed to explore this subject. A quantitative electronic dipstick reader measuring the concentrations of T-2 in urine could improve the dipstick test performance. It also could open new opportunities for further research into PEP diagnosis, as well as for the assessment of OD development.

3.4.5 STRENGTHS AND LIMITATIONS OF THE STUDY

Some strengths and limitations of the study are worth mentioning.

Studies I and II are retrospective in their nature, which naturally affects their results. However, there are only a few randomised studies of the endoscopic treatment of BDI, and the number of patients in them is smaller. Our study of 71 Amsterdam type A cases is noteworthy due to the size of the cohort, but the study's retrospective setting creates some limitations. Since the treatment modality was chosen by an endoscopist based on personal experience and not a randomly, there may be a bias in the study results. Furthermore, the exact time of fistula closure is unclear, because we did not follow the fistulas with serial repeat cholangiograms. Additionally, the grading of BDI according to Amsterdam classification and of the LG and HG leaks were conducted retrospectively, exploring cholangiograms and patient records.

Our cohort in study II, exploring the effects of diclofenac in the prevention of PEP in 2,000 patients is much larger than any published RTC and even some of the meta-analysis of this subject (Yaghoobi et al., 2013, Sun et al., 2014, Yuhara et al., 2014, Dai, Wang & Zhao, 2009). Due to the retrospective nature and inclusion criteria of the study, the study groups have some differences in their characteristics. More trainees were involved in ERCP in DG than CG. However, all the procedures were performed the same way and under the supervision of the same four experienced endoscopists who conducted most of the ERCPs. Trainees performed the easiest procedures, and their risk of PEP remained low (1.8-2.3%). Analyses of the 1,742 patients whose procedures were performed by the experienced endoscopists revealed that trainee involvement had no effect on the results: the PEP risk for all subgroups of patients were same in the DG and CG in whole cohort. Furthermore, more ES was done in the CG than in DG, and since pancreatic ES is a known risk factor for PEP, this also proved indirectly the diclofenac's inefficiency.

Study III, which examines the performance of the dipstick test in the diagnosis of PEP, is the largest to date. In addition to the study's size, our strength lies in the inclusion of all positively tested pre-ERCP dipstick patients in the analysis. Our study is the first to explore the dipstick test in clinical use in an endoscopic unit and to use the newest consensus criteria for PEP. It is a limitation, despite the size of the study, that we did not have significantly more PEPs than in previous studies and no cases of severe PEP. Some dipstick tests were lacking, mainly because 21% of patients were discharged from the hospital within a few hours after ERCP. This also complicated the analyses.

Study IV is a prospective study, and, to the best of our knowledge the largest of the performance of SPINK1, T-1 to 3 and trypsin-2-AAT in the diagnosis of SAP. It is also the first study of studied markers that has used the revised Atlanta criteria to distinguish between moderately severe and severe AP, and to explore the development of SAP in patients presenting without OD on

admission. A limitation of study IV is the limited number of SAP patients. Furthermore, the number of patients who developed SAP after admission might have been larger. However, our cohort of 29 SAP patients, including 21 patients who developed SAP after admission, is larger than in studies of other markers predicting SAP (Maksimow et al., 2014, Nieminen et al., 2014, Pantile et al., 2016).

3.5 CONCLUSIONS

I) ES is equal to ES and stenting in the treatment of Amsterdam type A bile duct leaks.

II) Rectal diclofenac shows no effect on the prevalence of PEP in a centre with a low risk of PEP.

III) A negative urine T-2 dipstick test rules out PEP 4 h after ERCP; a positive test with abdominal pain symptoms reveals PEP to high degree of accuracy.

IV) SPINK1 is an independent predictor of SAP at the time of admission to the hospital.

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